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THE DEVELOPMENT OF THE JAPANESE BLOOD-FLUKE, *SCHISTOSOMA JAPONICUM* KATSURADA, IN ITS FINAL HOST.*

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PLATES I TO IV.

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I. COMPARISON OF THE CERCARIA OF *Schistosoma japonicum* WITH THE ADULT.

Since the discovery of the intermediate host of *Schistosoma japonicum* by Miyairi and Suzuki in 1913, our knowledge of the life-cycle of the human blood-flukes has been very greatly increased. Very little is known, however, of the development after the cercaria enters the final host. The studies recorded in this paper were undertaken with the hope of filling in, to some extent, this gap in our knowledge in the case of *Schistosoma japonicum.* In order to point out the problems involved in this investigation, I will make a brief comparison between the cercaria of this species and the adult.

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* A contribution from the Department of Medical Zoölogy of the School of Hygiene and Public Health of the Johns Hopkins University.

The cercaria of *S. japonicum* (Text Figs. 1 and 2) * is very highly specialized for that critical period of intense activity from the time that it leaves the shelter of its snail intermediate host until it has penetrated safely through the skin of its final mammalian host.

During this period two demands are made upon the activities of the cercaria. One of these is for locomotion, both freely in the water and on a surface. For this activity the cercaria has a well developed muscular system, a strong tail, a ventral sucker which can be firmly attached, and a method by which it can take hold with its anterior end. Details of the methods of locomotion of the cercaria of *S. japonicum* have been reported in a previous paper (Cort, 1919, 502–505). The second demand that the cercaria must be prepared to meet is that of penetration through the thick skin of the mammalian host. The whole structure and activity of the cercaria is modified profoundly for this activity (Cort, 1919, 496–502). In fact, it has become essentially a mechanism for skin penetration. There have been developed very large cephalic glands (Text Figs. 1 and 2, *cg*) for dissolving tissue. The musculature of the body, and especially of the oral suckers, is definitely adapted for penetration, and the arrangement of the spines is an admirable adaptation for this purpose. This unusual development of the adaptive larval characters, especially the enormous size of the cephalic glands, has kept in a very undeveloped condition the primordial adult characters. The extreme immaturity of the cercaria is indicated by its small size, the slight development of its postacetabular region and the very rudimentary character of its nervous, excretory, digestive and reproductive systems

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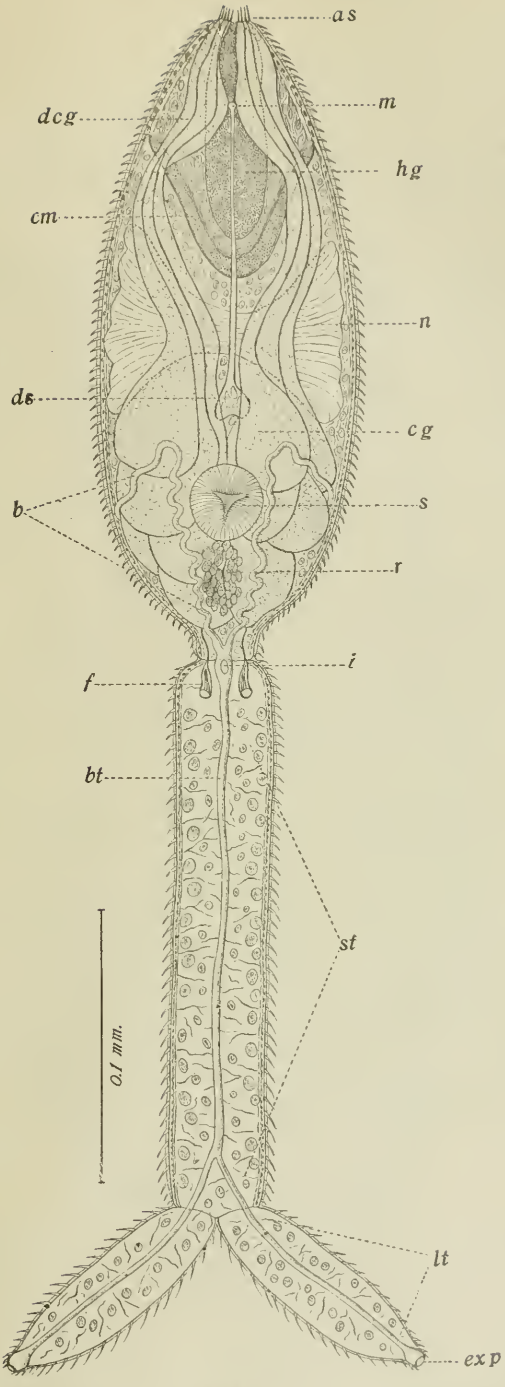
* In my paper on the cercaria of *S. japonicum* (Cort, 1919, 501) I described and figured forward pointing spines around the openings of the cephalic glands, which I believed aided the cercaria in penetration. Faust (1919, 168, Figs. 4 and 5) demonstrated in the cercaria of *S. mansoni* and related forms, that these spines on the anterior end of schistosome cercariae are hollow in the center, cap the openings of the cephalic glands, and that the secretions of these glands pour through the hollow spines. I have been able to confirm Faust's observations on the cercaria of *S. japonicum.*



TEXT FIG. 1. Cercaria of *S. Japonicum*, ventral view; *as*, anterior spines; *b*, excretory bladder; *bt*, excretory bladder of tail; *cg*, cephalic glands; *cm*, circular muscles; *dcg*, ducts of cephalic glands; *ds*, digestive system; *exp*, excretory pore; *f*, flame cell; *hg*, head gland; *i*, island in excretory bladder; *lt*, lobe of tail; *m*, mouth; *n*, nervous system; *st*, stem of tail; *s*, ventral sucker. (From Cort, 1919.)

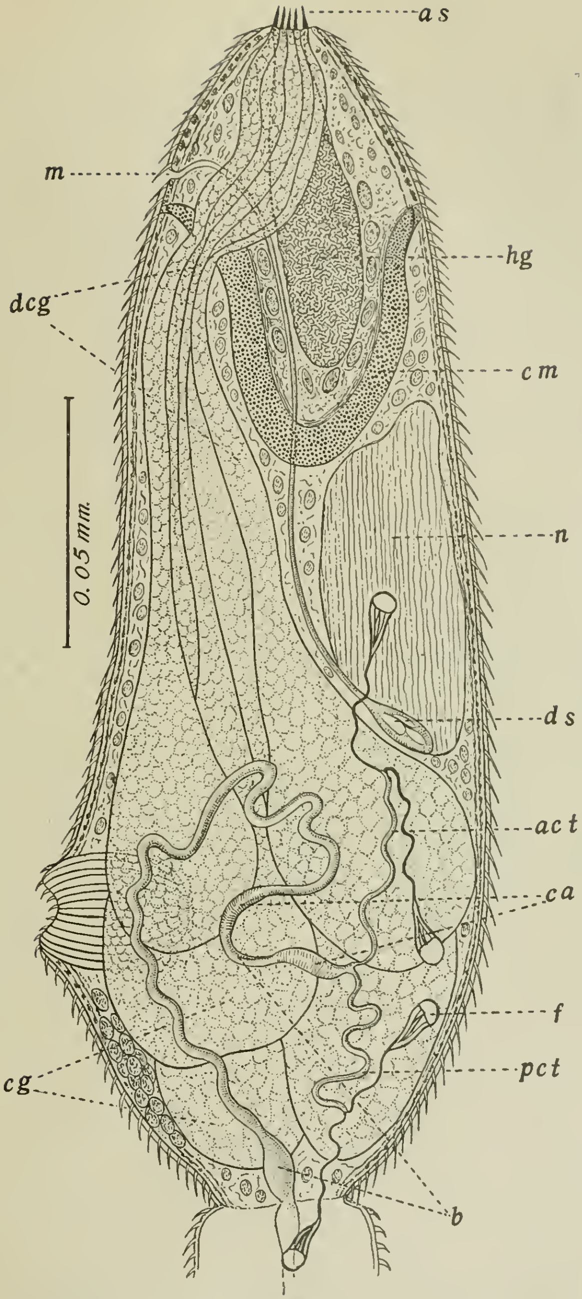
(Text Figs. 1 and 2). Finally, the sexual dimorphism which is such a striking characteristic of the adult has not been demonstrated in the cercaria.

The contrast in structure between the cercariae and the adults of *S. japonicum* (Figs. 11 (F) and 19 (M) is so striking that practically no similarity whatever can be made out between the various organs, unless the intermediate stages in development are known. The structure of the adult of this species is dominated by sexual dimorphism and adaptations for reproduction. The shape of the male is fundamentally modified from the general distome condition by the development of the gynaecophoric canal for holding the female in copulation. The female, on the other hand, has a very elongate, narrow body. These modifications have come about chiefly by changes in the postacetabular region of the body which are correlated with the gradual development of the reproductive organs.

The morphological changes which are necessary to produce the adult of *S. japonicum* from the cercaria are truly remarkable. The highly developed adaptive larval characters are lost soon after penetration. The length of the body must be increased fifty to even over one hundred times. The nervous and excretory systems already functioning in the cercaria must be greatly extended to meet the requirements of this rapid increase in size. The digestive system which is not yet functional in the cercaria must be organized for its activities and greatly increased in length, to keep pace with the extending body. The whole complexity of the reproductive system must be gradually evolved from the small group of nuclei, which are the primordia of this system in the cercaria (Text Figs. 1, *r*), in some individuals becoming male organs and in others female. Finally, the development of the secondary sexual characteristics which differentiate the sexes early, and later produce extreme sexual dimorphism, goes hand in hand with the development of the reproductive systems.

II. Material and technique.

The problems outlined above are interesting not only, because the changes from the cercaria to the adult in *Schistosoma japonicum* go on in the human body, but also from the more general biological viewpoint, since our knowledge is very inadequate of this stage in the development of the whole group of digenetic trematodes. An opportunity to attack this problem presented itself when on June



Text fig. 2. Cercaria of *S. japonicum,* side view; letters, as in Text fig. 1; also *act,* anterior collecting tube; *ca,* ciliated areas; *pct,* posterior collecting tube. (From Cort, 1919.)

23, 1919, I received from Dr. Sadao Yoshida of the Osaka Medical College, a package containing a large number of dried specimens of *Blanfordia nosophora* (Robson), the intermediate host of *S. japonicum.* These snails had been collected on May 28 from an area where Japanese schistosomiasis is prevalent. About 85 per cent. of them became active when placed in water, and about 4 per cent. of the living snails were found to harbor the cercariae of *S. japonicum.*

During the evening of the same day on which this material was received, a tablespoonful of the dried snails was placed in each of three small glass aquaria in water, at a depth of about two and one half inches. On the following day, it was found that almost all of the snails had become active and that the water was swarming with cercariae.

At 4 P. M. of this day, June 24th, thirteen white mice were placed in the three aquaria. The depth of the water was such that they could rest their hind legs on the bottom and just keep their heads above the surface of the water. The mice were kept in the water about twenty-five minutes. The following morning, seventeen hours after the first exposure, the mice were again placed for about twenty minutes in water, containing the active cercariae from four infected snails which had been crushed.

The first of the mice was examined forty hours after the first exposure to infection, and three young worms were found in the blood. The other mice were examined on the tenth, twelfth, fourteenth, sixteenth, eighteenth, nineteenth and twenty-first days after the first exposure. In all these mice large numbers of the worms were found in the veins of the liver. In fact, the mice were so heavily infected that after the sixteenth day they began to die, and it was barely possible to keep the last one alive until after the twenty-first day. In addition to the material from these thirteen mice, there was also available for comparison a number of specimens taken from mice thirty days after infection.

To recover the young worms the livers of the mice were carefully teased apart in normal saline, and the worms picked out with a small pipette. In the earlier examinations it was necessary to search carefully with a microscope to find the young schistosomes. On the later days they had grown to such an extent that they could be seen with the naked eye. The worms were killed and fixed by pouring suddenly over them a hot saturated aqueous solution of corrosive sublimate containing 2 per cent. of acetic acid. This method proved satisfactory since it caught the worms well extended. My observations were made from toto mounts prepared from this material.

The material available for study gave me a series of developmental stages from my smallest form, from the veins of the liver, 0.24 mm. in length, up to sexual maturity and copulation. A detailed study of that interesting period of reorganization from the cercaria to this smallest form will have to be left for future investigation. Since my studies were made on preserved material I have no data on the development of the excretory system. The present paper then includes a study of the changes in size and shape of the body, the changes in the suckers, the growth of the digestive system and the development of the reproductive organs and the secondary sexual characters which occurred in the veins of the livers of my experimental mice.

In addition to the data from my studies of the material discussed above, I shall include in this paper an account of all the phases in the life cycle from the free cercaria to maturity. This includes a discussion of the question of the time of determination of sex and possible sexual dimorphism in the cercaria, the method of penetration into the final host, the course of migration in the final host, and the early stages of development not included in my own studies. Most of the literature on these subjects is in the Japanese language. These publications were made available to me through the kind help of Dr. S. Yokogawa, of the Medical College of Formosa, and of Mr. Ibara, a graduate student in Johns Hopkins University who translated for me a part of Miyagawa's 1916 paper. I wish also to thank Miss Ethel Norris for her careful work in the preparation of the drawings included in this paper.

III. THE DETERMINATION OF SEX AND THE BEGINNING OF SEXUAL DIMORPHISM.

Since sexual dimorphism can be seen at a very early stage in the development of the larval schistosome in the final host, it seems perfectly evident that sexual differentiation is already present in the cercaria stage. From this consideration the question naturally arises whether there is not really present a sexual dimorphism in the cercaria stage. Altho many workers have made studies and measurements of the human schistosomes no one has reported such differences. I have myself, examined a number of cercariae of *S. japonicum* with this point especially in mind, without noting any dimorphism. Dr. S. Yokogawa also informs me that he has made an extensive series of examinations and measurements of this cercaria in an attempt to find sexual differences, without success. Since the cercariae of the human schistosomes are very small and can extend and contract their bodies to an unusual extent, slight size differences might escape notice in the living specimens and be difficult, if not impossible, to detect in measurements of preserved material. Further, these studies have been made from cercariae obtained from infected snails which had been crushed to free the cercariae. Such material would contain various stages in the development. Therefore variations in size, due to differences in the developmental stages of the specimens studied, might obscure a slight sexual dimorphism. I believe that if careful measurements were made of large numbers of cercariae after their escape from the snails, it might be possible to demonstrate size dimorphism between the sexes of this species.

Recently, in some studies on a species of schistosome cercaria with eyespots, from *Planorbis trivolvis*, from Douglas Lake, Michigan, I have been able to demonstrate two distinct size types. This difference in size came to my attention first when I found that the curve plotted from the measurements of the cercariae which had escaped from a number of infested snails was distinctly bimodal. More extensive studies showed that the cercariae of this species fell into two distinct size groups. I further found from measurements of the cercariae from eleven infested snails that in the cercariae coming from a single snail only one of the size types was represented. The difference in size was so great betwen these two types that it could be recognized with the naked eye, when free swimming cercariae of the two types were placed in separate bottles. Measurements of the length of the body of the larger type showed a range of variation from 0.234 mm. to 0.28 mm., while in the smaller type, the range was from 0.207 mm. to 0.24 mm. Other measurements of the body and tail, which in this species is unusually large, showed like differences. The adult into which this cercaria develops is not known, although unsuccessful attempts were made to introduce it into ducks and rats. An analysis of its structure, however, places it near to the human schistosomes, in the family Schistosomidae. This relationship means that in all probability in the adult stage of this species the sexes are separate. I therefore, interpret the size differences in this species of cercaria as a sexual dimorphism. If this view is accepted, the fact that in one infested snail only one of the types of cercaria is represented, immediately becomes very significant. A more detailed account of the dimorphism of this species of cercaria will be published later in connection with a study of its structure and activities.

In this connection must also be cited the work of Tanabe (1919) on *Schistosoma japonicum.* This author found that in twenty-six out of thirty-one cases, when the cercariae from a single snail were used in infecting experimental animals, all the individuals developed were of the same sex. Dr. S. Yokogawa has given me permission to use in this connection, the results of some of his unpublished experiments along this line, which were performed several years ago. He found that when a cat, dog, or rabbit was infected with the cercariae from a single snail that worms of only one sex would develop. He also found that in these cases the worms would not develop to maturity. These two workers have developed independently the same hypothesis to explain the results of these experiments.

According to this hypothesis sex in the schistosomes is determined in the fertilized egg, and all the cercariae coming from a single miracidium are of the same sex. When all the individuals derived from the cercariae from a single snail were of the same sex, it would follow that the infestation in this snail was from a single miracidium or two or more miracidia of the same sex. In those cases where both sexes came from the same snail, this snail must have been originally infected with two or more miracidia representing both sexes. Now my findings recorded above in regard to dimorphism in a species of schistosome cercaria, and the presence in one snail of only one of these types lends further support to this hypothesis. Further, since in the life cycle of *S. japonicum,* the miracidium and the mother sporocyst are the only stages derived from a fertilized egg, it is in these stages that sex differentiation would theoretically be expected. Up to the present time, however, no one has examined these stages to determine whether they show a sexual dimorphism.

IV. PENETRATION OF THE CERCARIAE THROUGH THE SKIN OF THE FINAL HOST.

The structures of the body of the cercaria of *S. japonicum* which make possible the penetration through the thick skin of the mammalian host have been discussed above. It has also been clearly established (Fujinami and Nakamura, 1909; Katsurada and Hasegawa, 1910; Matsuura, 1909 and Fujinami, 1914) that infection with *S. japonicum* is produced by pentration of the cercariae through the skin or mucous membrane. Infection by way of the digestive tract has been shown to be impossible (Tsuchiya, 1910; Fujinami and Nakamura, 1911; and Miyagawa, 1912a), since the cercariae cannot resist the action of the digestive juices. However, the infection with drinking water is possible, since the cercariae taken into the mouth may penetrate through the mucous membrane of the mouth and pharynx. Penetration through the skin of the cercaria of *S. japonicum* has been discussed most fully by Narabayashi (1914, 1916). He finds that the cercariae can penetrate through wet skin at any point where they become attached, although they actually seem to enter most frequently by way of the hair follicles. Usually, they entered singly, altho sometimes two or three were found at the same place. In examinations of the skin from one to twenty hours after exposure to infection, the cercariae were found in the epidermis, dermis and subcuticula and sometimes in the deeper layers. The worms found in the skin tissues show various shapes and degrees of contraction. Usually, the tail is lost at the time of penetration through the skin, but in some cases it may be carried into the subcuticular layer. In one of my experimental mice I found a cercaria in the blood from the heart, with the tail still attached. The worms entering the skin seem to be attracted to the blood vessels, for soon numbers of them will be found in the capillaries, veins or lymph spaces of the dermis and subcuticula.

V. THE COURSE OF MIGRATION IN THE FINAL HOST.

The course which the larval blood flukes pursue in making their way from the skin to the blood vessels of the liver has been a matter of considerable controversy. Fujinami and Nakamura (1910) found the young parasites in the portal vein of a rabbit, three days after exposure to infection, and suggested that the course of migration in the body was probably by way of the blood stream. Miyagawa (1912; 1913; 1913a; 1913b) found the young flukes in the femoral and saphenous veins, in the hilus of the lymph glands, in the thoracic duct, and finally in the heart of experimental dogs. Later this same author (Miyagawa, 1916) also found the parasites in the capillary vessels of the lungs, kidney and intestinal walls, and in the left side of the heart. From his studies, he came to the conclusion that the young blood flukes might travel to the right side of the heart either by the veins or lymph vessels, be pumped into the lungs, pass the capillaries of the lungs and return to the heart. From the left side of the heart they would be carried into the systemic circulation, and must again pass through capillaries to reach the mesenteric veins and the portal vein. Narabayashi (1914, 1916) agreed with Miyagawa that the above course is sometimes followed by the worms. He concluded, however, that the majority of the young blood flukes after reaching the lungs leave the blood vessels and migrate to the liver through the tissues. Strong evidence for this course has been furnished by the extended researches of Narabayashi (1916). This author infected large numbers of very young mice with the cercariae of *S. japonicum* and made serial sections of their whole bodies. He found that the majority of the cercariae, after passing through the skin, entered the veins and that only a few reached the heart by way of the lymph vessels, since most of those that got into the lymphatic system died in the lymph glands. He found them only rarely in the capillaries of the lungs, in the left heart and in the blood vessels of the kidneys. The larger number he found migrating through the connective tissue of the posterior mediastinum, along the large blood vessels and nerve trunks, while a smaller number was encountered also in the anterior mediastinum. Still others were found in the pleural cavity. He also found them in the tissues of the diaphragm and on the surface of the liver, but never in the hepatic, splenic and mesenteric arteries. Recently Suyeyasa (1919) repeated Narabayashi's experiments and came to the same conclusions. It seems probable, therefore, from these studies, that the usual course of migration is for the larval blood flukes to leave the blood vessels of the lungs, to pass through the diaphragm, and to enter the liver from its surface.

It is probable from the character of the migratory course described above that there would be considerable variation in the time necessary for the migration. It would be expected, therefore, that, in the course of a single infection larvae would keep entering the liver for a considerable length of time. That this actually happens is suggested by my finding very small larvae, less than 0.3 mm. in length, in the liver ten, twelve, and up to eighteen days after exposure to infection. This point will be discussed more in detail in a later section.

VI. THE MORPHOLOGICAL CHANGES IN THE DEVELOPMENT OF *S. japonicum* IN ITS FINAL HOST.

1. *Historical account.*

Up to the present time a detailed analysis of the morphological changes from the schistosome cercaria to the adult has not been made.

I find, however, that a number of authors have made observations on this stage of development. Most of these observations have been made on the development of *S. japonicum,* and most of them are included in papers written in Japanese. Leiper (1915) has figured a series of stages in the development of *S. haematobium* in its final host, but he does not describe or compare his stages, nor does he distinguish the sexes. Lutz (1919) in his discussion of *S. mansoni* gives very few data on this stage in development.

I will not attempt to discuss in detail all the observations which have been made on this stage in *S. japonicum,* but merely bring out the significant points in the studies of the various investigators. Miyagawa (1916) in his part of the Japanese monograph on *S. japonicum* (Fujinami, Tsuchiya and Miyagawa, 1916) discusses in some detail the work which has been done on this stage of development of *S. japonicum.* My discussion of the Japanese work is based upon reviews, upon a translation of the part of Miyagawa's paper dealing with this subject and upon discussions with Dr. S. Yokogawa.

Miyagawa (1916, 17) stated that in the larvae examined after entering the skin the ''head gland'' was found to be reduced in size, pressed to one side, and that later it disappeared. He also notes that in such worms the contents of the cephalic glands are much reduced in amount, and that these organs soon disappear.

No detailed morphological studies have been made of the development of the worms in the tissues, or during their migration to the liver. Miyagawa finds that the size of the young worms taken from the skin and the peripheral veins varies from 0.038 to 0.15 mm. in length, and from 0.012 to 0.068 mm. in width. Since the smaller limits of his ranges of variation are only a fraction of the size of the cercaria itself, it is evident that some of the specimens which he studied must have been extremely contracted, on account of the technique used in preservation. Narabayashi (1914) found young blood flukes in the skin having a length of 0.143 mm. and a width of 0.034 mm. Fujinami and Nakamura (1910) found a young fluke in the portal vein of a rabbit, three days after infection, with a length of 0.15 mm. and a width of 0.037 mm. Miyagawa (1916) also reports a worm in the portal vein of a dog, five days after exposure to infection, which measured 0.5 mm. in length and 0.058 mm. in width. Katsurada (1914, 372, Fig. 2) describes and figures a young stage 0.438 mm. in length and 0.12 mm. in width, taken from the liver of a dog on the seventh day after infection. This figure is evidently of a male of about the stage of my Fig. 3 (*M*). The oral sucker in this specimen had become definitely rounded off and the intestinal ceca are well developed, and distended with a dark brown material, altho not yet united. Miyagawa (1916) also notes that the spines in the young worms up to 0.3 mm. can be very clearly distinguished, but that it is very difficult to see the spines, even in the preacetabular region in a specimen 1 mm. in length. He further records that at the 0.3 mm. stage the oral sucker has changed from the peculiar condition of the cercaria to a well rounded muscular organ, with a good sized mouth cavity.

Miyagawa (1916) also gives some interesting information on the early development of the digestive system. He finds that in the larvae taken from the skin the digestive system is already functioning, since it can be seen clearly and is distended with dark staining material. In the early stages it appears like a distended bag which is connected with the oral sucker by the narrow esophagus. A little later, the intestinal ceca develop, and in forms 0.2 to 0.3 mm. in length, the shape is like a horse shoe. A little later the ceca unite, and the common portion reaches almost to the posterior end of the body. Miyagawa offers a very interesting observation on the relation of the liver to the development of the blood fluke. He differs from Narabayashi's (1916) position that the liver is simply a station in the course of migration of the worms, and believes that it is the natural location for the worms and that only in it can growth go on normally. Further, it is only when females deposit eggs that the copulating worms work their way out from the blood vessels of the liver to the mesenteric veins.

According to the Japanese workers the reproductive organs become differentiated late, but sexual dimorphism appears early. Fujinami and Nakamura (1911) were able to distinguish the sexes in specimens 0.5 to 0.7 mm. in length, which had developed in dogs. According to these authors, the body of the male becomes early broader than the female, especially in the region of the branching of the intestinal ceca. Also, the suckers are somewhat larger in the male than in the female. They further find that there is a distinct difference in the region where the intestinal ceca unite. In the male the region between the intestinal ceca, just in front of the point of union is a long narrow triangle, while in the female this region, where the ovary develops is even at an early stage wider. Miyagawa (1916) notes that the differentiation of the reproductive organs comes comparatively late, and that they can not be clearly distinguished until a length of about 1 mm. is reached. He finds copulating worms twenty days after infection.

2. *Growth in size.*

One of the most striking changes in the development of *S. japonicum* in its final host is the remarkable increase in size. In the published records the sizes given for the body of the cercariae vary from 0.09 mm. to 0.23 mm. These variations for the most part depend on whether the measurements were made from living or preserved specimens, on the state of contraction, and on the method of preservation used. Since the body of a well extended, preserved specimen measures about 0.15 mm., I will use this size for comparison of the cercaria with further stages of development. The largest size which I have seen recorded for the adult of *S. japonicum* was 26 mm. in length for the female, and 22.5 mm. for the male. These measurements were the maximum found by Tsuchiya for living specimens, taken from man, and by Katsurada (see Katsurada, 1914, 366) from living specimens 21 months old, taken from a dog. To attain this size an increase in length of about 130 times over the cercaria is necessary.

The growth of *S. japonicum* in the final host may be divided into three periods, i.e. (1) from the penetration of the cercaria until the young worm reaches the blood vessels of the liver, (2) from the time of reaching the liver until sexual maturity, and (3) from sexual maturity to maximum size.

There is but little increase in size in the young worm which has not yet reached the liver. The three specimens which I found in the blood of the first mouse, examined forty hours after infection, appeared somewhat shrunken from the loss of the secretions of the cephalic glands, but showed no increase in length over the free cercaria. One of these larvae was very remarkable in having the tail still attached to the body.

My smallest specimen from the livers of the mice was 0.22 mm. in length and 0.1 mm. in width. This specimen was recovered 12 days after infection. Specimens almost as small were recovered on the 10th day, the 14th day and the 18th day after infection (see Table I). It can be concluded from these results that there is but little increase in size until the worms reach the liver, since such specimens as have been found in passage to this organ are very small and the smallest forms found in the liver are but little larger than the cercariae.

Further, the finding of very small specimens in the liver, as late as 18 days after infection, might be explained by considering that such a form had been delayed in its passage to the liver, and that favorable conditions for growth in size are not found until this organ is reached.

During the second period there is by far the most rapid increase in size. As in other trematodes, *S. japonicum* reaches sexual maturity long before the maximum size is reached. My earliest record of copulation was nineteen days after infection. Individuals in copulation might be considered sexually mature, altho in the youngest pairs the uterus of the female did not yet contain eggs. The male in the youngest copulating pair which I measured, twenty-one days after infection, [Fig. 10 (F.) and 17 (M.)] had a length of 5.6 mm. and the female of 4.2 mm. It is interesting to note that in this early stage of copulation the female is considerably shorter than the male, while later the size relation is reversed. From these figures it can be seen that in the period of development in the liver of the mouse, up to sexual maturity, there can be an increase in less than twenty days from about 0.2 mm. to 0.3 mm. up to 5.6 mm. in the male, and 4.2 in the female, or an increase in length of 28 times and 21 times respectively. A comparison of the largest individual found on each of the days on which examinations were made indicates approximately the rate of growth during this period (see Table I). The largest individual, twelve days after infection, was 0.79 mm. in length, which is about five times the length of the cercaria, and about three

TABLE I.

Range of size in specimens found on different days.

	Number of days after first exposure to infection.						
	12	14	16	18	19	21	30
Male—							
Number of specimens measured*	10	8	9	7	13	13	10
Smallest	0.22	0.54	0.28	0.22	0.40	0.50	2.21
Largest	0.79	0.97	2.02	2.34	3.8	5.3	8.8
Female—							
Number of specimens measured*	12	15	10	13	10	10	10
Smallest	0.22	0.27	0.50	0.63	0.53	0.64	3.87
Largest	0.77	0.99	1.94	1.89	2.65	4.2	8.27

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* This does not represent the total number of specimens examined, since only those which were apparently near the upper or lower range were measured for this table.

times the length of the youngest form which I have found in the liver. From the twelfth to the nineteenth or twenty-first days, when copulation takes place, the increase in size is still more rapid, In these seven to nine days the largest individuals show a gain in length of from five to seven times. It is during this period that the increase in size is most striking, since the worms which are barely visible to the naked eye in the earlier stages, on the later days are easily seen and picked out. In fact, the growth was so rapid that it could be noted with the naked eye at the two day periods. It is also during this period that in my heavily infected mice the worms clogged the blood vessels of the liver, and seriously affected their hosts.

The third period of growth of *S. japonicum* in its final host extends from sexual maturity until maximum size is attained. During this period, the growth is slow and extends over a long period of time. We have no very exact information in regard to the actual rate of growth during this period or how long growth may continue. In my experimental mice, from the 21st to the 30th day, the growth in size was much less than in the corresponding time in the second period (Table I). My experiments give me no further data in regard to growth during this period. Katsurada (1914) records specimens in mice which reached the size of 8 mm. in the male and 11 mm. in the female, 62 days after infection.

Further data on growth during the final period of development may be obtained from Tables II and III which I have copied from Katsurada (1914, 365 and 366). The data included in these two tables give some idea of the rate of growth during the third period, and the length of time that growth continues.

The records of the specimens taken from the dogs 82 days, 10 months and 21 months after infection, are especially to the point. Full size seems to have been attained in 21 months, since the largest specimen at this time is as large as the largest individuals ever recorded.

Katsurada (1914, 365) comes to the following conclusion in regard to the size-relation in *S. japonicum*.

> Die Grösse der Tiere hängt von mancherlei Umständen ab, z. B. von ihren Entwickelungsstadien, von ihrer Individualität, von der Art des Wirtes und von sonstigen Verhältnissen. Nach unserer Beobachtung kann ein kleines Tierchen, dessen Körperlänge noch nicht ganz 5 mm. beträgt, schon reif sein.

His contention that the size of the host influences profoundly the size of the parasite is very interesting.

TABLE II.

Tabelle über Körperlänge des Schistosomum japonicum.

Autoren	Katsurada (1904).		Tsuchiya (1906).		Fuyinami (1907).		Katsurada (1909).		Katsurada (1911).		Katsurada (1913).	
Geschlecht	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Durchschnittl. Körperlänge (mm.)	10.43		16.1	21.8	16.0	17.8	10.92	14.5	8.0	11.0	12.8	10.6
Grösster Längendurchmesser (mm.)	12.0	12.0	19.5	26.0	21.0	23.0	13.0	17.0			15.0	12.0
Kleinster Längendurchmesser (mm.)	7.0	8.0	9.5	16.0	8.0	12.0	9.0	11.0			11.0	8.5
Zahl der Würmer	8		19	8			13	4			5	5
Zustand der Würmer	Formalin-fixation		Frisch (aber 24 Std. n. d. Tod des Wirtes)				Formalin-fixation		Frisch		Spiritus-fixation	
Wirt	Katze		Mensch		Rind		Hund, gestorben, 82 Tage n. d. Ansteckungsversuch		Maus, getötet, 62 Tage n. d. Ansteckungsversuch		Hund, getötet, 10 Monate n. d. Ansteckungsversuch	

TABLE III.

Besonders gut entwickelte Schistosoma (sic) japonicum. Beispiel von 21 Monate alten Würmern—Wirt: männlicher Hund (Körpergewicht ungefähr 11 kg.)—frischer Zustand.

Geschlecht.		No.	Längen-durchmesser (mm.).	Breitendurchmesser (mm.).		Mundsaugnapf-durchmesser (mm.). Querdurch-messer.	Bauchsaugnapfdurchmesser (mm.).	
							Querdurch-messer.	Längendurch-messer.
Unpaarige Schistosomen	♂	1 dickster	21.0	1.5		0.604	0.604	0.604
		2	20.0	1.2		0.513	0.581	0.467
		3	22.5	1.0		0.513	0.627	0.593
		4 dünnster	12.0	0.7		0.513	0.570	0.534
				Vorderkörper	Hinterkörper			
	♀	1 Längster	26.0	0.125	—	—	—	0.114
		2 Kürzester	18.0	0.137	0.445	—	—	0.091
Paarige Schistosomen	I Paar	♂	19.5	0.627	0.8	0.456	0.536	0.570
		♀	23.0	0.103	0.422	—	—	0.114
	II Paar	♂	19.5	0.570	0.8	0.502	0.559	0.570
		♀	21.0	0.103	0.513	—	—	0.103
	III Paar	♂	19.0	0.513	0.8	0.456	0.522	0.513
		♀	23.0	0.137	0.456	—	—	0.091

An important point which requires further discussion is the range of variation of individuals recovered on any given day after infection (see Table I). While this variation is very clear as early as the 12th day, with a maximum size of 0.79 mm. and a minimum of 0.22 mm., it is most strikingly exhibited during the later periods. On the 16th to the 19th days numbers of young distomes were found below 0.72 mm. in length, while the maximum on the 19th day had run up to 3.8 mm. in the male and 2.65 mm. in the female. Especially interesting was the finding of a male individual on the 18th day only 0.22 mm. in length. This specimen was very much contracted and probably represents about the stage of Fig. 2 (M.). By the 21st day no very small individuals were found, altho the range of variation was still quite great. By the 30th day the smallest individual found in a large series of examinations was a male, 2.21 mm. in length.

Since the mice were infected twice, with a lapse of about 17 hours between exposures, a slight degree of variation could be attributed to this cause. This cause is, of course, entirely inadequate to explain the great variations noted in Table I on all the days on which examinations were made, and especially the finding of very small individuals as late as 18 days after exposure to infection. To adequately explain these variations two things must be postulated, i.e., first a variation in the time taken for the passage from the skin to the veins of the liver, and second, the necessity of any given individual reaching the liver before there can be much increase in size. From the description given above, of the course of migration of the larval blood flukes, it seems certain that there must be a considerable variation in the time which is taken by the parasites in reaching their destination. From the large numbers which were found in the livers of the mice by the 12th day it seems probable that most of the larvae complete their course by this time. The view that only in the blood vessels of the liver can the young schistosomes find conditions favorable for their growth, is supported by the fact that the worms which have been found outside the liver in experimental animals, had increased but little in size over the cercaria. It is my opinion, therefore, that the range in size of the individuals found in the livers of my experimental mice was due to differences in the time taken in reaching the liver, and that the small individuals found in the later periods after infection, represented forms which had been considerably delayed in their way to the liver. That there is a definite limit to the time which may be taken in making this course, is indicated by the fact that on the 30th day no small worms were found. Apparently all the worms, that were going to reach the liver had already arrived.

3. *Changes in shape.*

Probably the most remarkable of the changes in the development of *S. japonicum* in its final host are the changes in shape. In the cercaria (Text Fig. 1) unless in an extreme state of extension the length of the body is about three times its width. The length of the post-acetabular region is only about one-half that of the pre-acetabular, and the cross-section is round. The shape is but slightly different in my smallest specimen from the liver [Fig. 1 (F.) or (M.)], in which the length is about two and one half times the width. The length of the post-acetabular region is still less than the pre-acetabular and the cross-section is oval. Since the shape of the adult males and females [Figs. 11 (F.) and 18 (M.)] are so remarkably modified by the sexual dimorphism, the study of the changes by which these peculiarities are attained through a series of developmental stages is interesting and significant. The shape of the adults is so strikingly different from that of the cercaria that it is very remarkable that such changes could be accomplished in the short period of 18 or 19 days which it takes the developing fluke to assume the characteristic shape of the adult. These changes consist in a very great increase of the length in ratio to the width, an enormous increase of the post-acetabular region of the body, as compared with the pre-acetabular, and a gradual assumption of the secondary sexual characteristics which produce the sexual dimorphism of the adult. Since the changes in shape are very different in the male and the female, the sexes will be discussed separately.

The ratio of length to width increases very greatly in the female (Table IV) as can be seen by a comparison of figures 3 (F.), 5 (F.), 7 (F.), 8 (F.) and 10 (F.). In these forms, the ratios of length to width are as follows: 3 (F.) = 33:10; 5 (F.) = 20.3; 7 (F.) = 12:1; 8 (F.) = 18:1; 10 (F.) = 28:1.

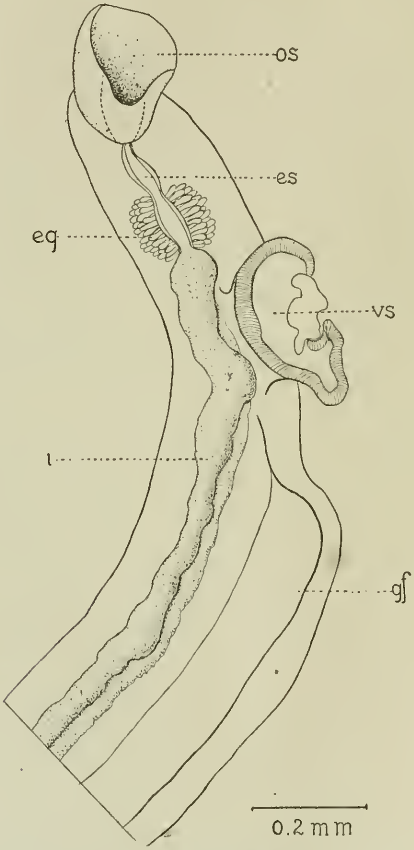
In the specimens, 30 days old, from my experimental mice this ratio varied from 50:1 to 90:1. The width in all these ratios was measured in the region of the ventral sucker. After the reproductive organs are fully developed, the post-ovarian region becomes considerably wider than the pre-ovarian. This is shown in Fig. 11 (F.), and Katsurada's measurements (see Table III) of three 21-month-old females, in copula, in which the ratio of length to width of the pre-ovarian region is about 200:1, while that in the post-ovarian region is only about 50:1.

The great increase in length of the female occurs almost entirely in the post-acetabular region and is correlated with the development of the reproductive organs and the extreme attenuation of the body. In my smallest form which can surely be considered a female [Fig. 2 F.)], the growth of the post-acetabular region has already brought the acetabulum in front of the middle of the body, and the pre-acetabular region is 48 per cent. of the total body length. In a series of females the percentage of the total body length occupied by the pre-acetabular region decreases rapidly with growth, as follows: Fig. 3 (F.) = 43 per cent.; Fig. 4 (F.) = 37.5 per cent.; Fig. 5 (F.) = 11 per cent.; Fig. 10 (F.) = 6 per cent. In the females of the thirty-day period this percentage has been reduced as low as 3 to 4 per cent. The pre-acetabular region also becomes distinctly narrower with growth.

Another change in the shape of the body of the female is an increase in thickness which changes the shape of the cross-section from an oval, in the early stages, to a circle in the later stages. This modification is completed at about the size of 7 (F.). When the body has become rounded in cross-section the young female worm comes to lie on the side and tends to curl up [See Figs. 8 (F.) and 9 (F.)].

In the males of *S. japonicum* the changes in shape are of an entirely different character from those in the female. In the male the length increases very much in ratio to the width, but not to the extent noted above, for the female. The changes in the ratio of the length to the width up to the time of the formation of the gynaecophoric canal are illustrated by the following series, i.e., Fig. 2 (M.) = 30:11; Fig. 4 (M.) = 44:13; Fig. 7 (M.) = 5:1; Fig. 9 (M.) = 6:1. After the formation of the gynaecophoric canal this change in ratio is even more rapid, as is shown by the following series: Fig. 11 (M.) = 7:1; Fig. 14 (M.) = 9:1; Fig. 17 (M.) = 16:1.

Also the increase in length of the post-acetabular region is not quite as great, as in the female. In a typical male series, the percentage of body length which is occupied by the pre-acetabular region, is as follows: Fig. 5 (M.) = 40 per cent.; Fig. 7 (M.) = 31 per cent.; Fig. 10 (M.) = 22 per cent.; Fig. 14 (M) = 21 per cent. As development proceeds, the pre-acetabular region becomes distinctly set off from the rest of the body (Fig. 10 (M.) and Text fig. 3), and becomes round in cross section, not sharing in the flattening of the post-acetabular region, in connection with the formation of the gynaecophoric canal.



TEXT FIG. 3. Anterior region of half-grown male specimen of *S. japonicum*, to show an early stage in the development of the gynaecophoric canal; *eg*, esophageal gland; *es*, esophagus; *gf*, fold of gynaecophoric canal; *i*, intestine; *os*, oral sucker; *vs*, ventral sucker.

As noted above, from an early stage in development, the post-acetabular region of the male is distinctly wider than in the female. Also, the body in this region tends to become thinner [Fig. 4 (M.), 5 (M.), 10 (M.)]. In the earlier stages the post-acetabular region is not set off from the rest of the body. It is only when the length of the young distome approaches 2 mm. that the characteristic shape of the post-acetabular region begins to be assumed. At this stage the post-acetabular region is set off distinctly from the pre-acetabular, the widening coming just behind the base of the acetabulum, which at this stage juts out considerably from the body.

Just after this stage, the sides of the thin post-acetabular region begins to grow up to form a trough [Figs. 11 (M.), 13 (M.), 14 (M.) and Text fig. 3]. As soon as this growth comes about, the shape of the male becomes such that it tends to turn over on its side and often to curve toward the dorsal side [Fig. 13 (M.)]. Soon the folding over becomes so great that the opening of the groove is almost closed by the sides of the body [Fig. 14 (M.)]. At this stage, the canal is almost completely formed, and is soon ready to enclose the female. During the course of this development, the body has become very thin.

4. *Development of the ventral sucker or acetabulum.*

In my description of the cercaria of *S. japonicum,* the following statement is made in regard to the ventral sucker (Cort, 1919, 494). "The ventral sucker is located at a point about three fourths of the distance from the anterior to the posterior end of the body. It is circular in ventral view with a depth considerably greater than its diameter. In the living animal the ventral sucker can be protruded so that it has almost the appearance of a proboscis. The sucker functions strongly in locomotion and is able to hold the cercaria against considerable pressure." As shown in Text fig. 1 the ventral sucker of the cercaria has only about one fourth the length of the oral sucker and one third its width. It has a diameter varying from about 0.018 mm. to 0.022 mm. Contrast with this the large size of the ventral sucker of the adults, especially the males, or even of the copulating pairs [Fig. 10 (F.) and 17 (M.)]. Katsurada (1914, 366) gives measurements of the ventral suckers of specimens taken from a dog 21 months after infection (See Table III). In this material the diameter of the ventral sucker varies, in the male from 0.5 mm. to almost 0.63 mm., and in the female from 0.091 to 0.114. This difference in the size of the ventral sucker of the male and female is apparent at a very early stage, and is one of the ways in which the sexes can be distinguished in the young worms [cf. Figs. 4 (F.) and 4 (M.)]. The stages in the development of the ventral sucker can be followed step by step in the females, and the increases in size at the various stages seen in Table IV. The change in position of the ventral sucker, due to the relatively great increase in length of the post-acetabular region has been discussed in another connection.

In my youngest form [Fig. 1 (M. or F.)], in which I am not sure of the sex, the ventral sucker has almost doubled in size over the cercaria, having a length of 0.034 mm. and a width of 0.038 mm. In Fig. 4 (F.) in which the length of the body is almost twice that of Fig. 1 (F. or M.) the ventral sucker has a length of 0.067 mm. and a width of 0.068 mm. Beyond this stage, the increase in size of the ventral sucker of the female is more gradual, and not in proportion to the increase in body length. This can be seen by comparing Fig. 4 (F.) with Fig. 8 (F.), in which the length of the body has been almost quadrupled with only a 50 per cent. increase in the size of the ventral sucker (See Table IV). Beyond this stage, there is very little further increase in the size of the ventral sucker, although the length of the body increases very greatly. This is shown by a comparison of 8 (F.), with a total length of 1.8 mm., and a ventral sucker with a diameter of 0.099 mm., and the female from Katsurada's first copulating pair (see Table III), in which the length of the body was 23 mm., and the diameter of the ventral sucker only 0.114 mm.

In the early stages of the development of *S. japonicum* in its final host the ventral sucker is like that of any young distome. As the female elongates, so that the ventral sucker comes to lie in the anterior third of the body, it becomes thinner and begins to be distinctly set out from the surface of the body [Figs. 6 (F.)]. At the stage shown in Fig 7 (F.) the ventral sucker is saucer-shaped and somewhat pedunculate, and by the time the female becomes circular in cross-section, it assumes the characteristic adult shape of a shallow saucer set on a short stalk [Fig. 8 (F.)].

In the male the great increase in the size of the ventral sucker can be seen from my series of figures [Figs. 2 (M.) to 17 (M.)], and by comparing the measurements in Table IV. Here, as in the female, the rate of increase in size is greater in the earlier stage of development, but continues until growth is completed. Therefore, in comparing Fig. 3 (M.) with Fig. 4 (M.) (See also Table IV) it will be noted that while the increase in length is only about 50 per cent., the size of the ventral sucker has been doubled, while in the growth from the stage of Fig. 4 (M.) to that of Fig. 7 (M.) the length has been almost doubled, while the increase in size of the ventral sucker has been less than 50 per cent. A still further slowing of the rate of growth is apparent from a comparison of Fig. 7 (M.) with Fig. 14 (M.) in which the length has been almost quadrupled, while the size of the ventral suckers has been only a little more than doubled (see also Table IV). This rate of increase becomes still less in the later stages. This can be clearly shown by a comparison of Fig. 14 (M.) with the adult male [Fig. 18 (M.)].

In the male the change in shape of the ventral sucker, during development, is even greater than in the female. At early stages in the male, the ventral sucker becomes very thin and flexible, as is shown by the irregularity of its shape, and it juts out from the body [Figs. 4 (M.) and 5 (M.)]. At an early stage in the formation of the gynaecophoric canal the ventral sucker has assumed the characteristic shape of the adult [Fig. 11 (M.) and Fig. 14 (M.)]. The ventral sucker is very thin and flexible, with the outline quite irregular and varying from saucer shape to cup shape. It is always set off from the body with a distinct stalk, and after the formation of the gynaecophoric canal it is set in the hollow formed between the rounded neck region and the anterior limits of the sides of the gynaecophoric canal (Text fig. 3).

There is, of course, a considerable degree of variation in the size of the ventral sucker in individuals of the same length. This variation, however, is not sufficient to invalidate the course of development as outlined above.

3. *Development of the oral sucker.*

The oral sucker of the cercaria of *S. japonicum* has been described in an earlier paper (Cort, 1919, 496 to 497), and is shown in Text figs. 1 and 2. The oral sucker at this stage has scarcely any of the permanent characteristics of the adult, and its structure is very much modified by the presence of adaptive larval characters, and by its relation to the activities of the cercaria in locomotion and penetration. The oral sucker of the cercaria occupies almost one-third of the body length, and the mouth and buccal cavity are very small (Text figs. 1 and 2, M.). The oral sucker of the adult [Figs. 11 (F.) and 18 (M.)] is entirely different from that of the cercaria. While it has increased very greatly in size, it has come to occupy only a very small fraction of the body length. The oral sucker has now assumed the shape of a funnel with a very large mouth and thin lips. The buccal cavity is funnel-shaped and small at the point where it joins the esophagus. The mouth opens on the ventral side, and the shape of the sucker and buccal cavity is asymmetrical. At the stages shown in Fig. 8 (F.) and Text fig. 3 the oral sucker has already assumed its characteristic adult condition. A comparison of these figures with the cercaria show how profoundly this structure has been modified in development.

In the smallest individual which I have figured [Fig. 1 (F. or M.)] the oral sucker has already changed fundamentally from the condition of the cercaria. The adaptive larval characters, i.e., the anterior spines, the ducts of the cephalic glands and the "head gland" have already disappeared. The mouth and buccal cavity have increased in size, the latter being already funnel-shaped. The characteristic ventral position of the mouth is apparent at this stage, establishing definitely its relation to the mouth, as shown in the cercaria [Text fig. 1 (M.)]. While the diameter of the mouth at this stage is greatly increased over the condition of the cercaria, it is as yet only a little over one fourth the diameter of the oral sucker. The oral sucker in this specimen occupies slightly over one fourth of the body length, the length of the body in this specimen being 0.24 mm., and the length of the oral sucker 0.063 mm. The oral sucker at even this early stage is much more definitely set off from the rest of the body than in the cercaria, and its muscles are developed in relation to the sucking activity of the mouth and buccal cavity.

Early in the development there is a greater proportional increase in size in the oral sucker of the male than of the female. In fact, the easiest way to distinguish the sexes in the earlier stages is by a comparison of the size of the oral suckers. This difference is shown clearly by comparing the increases in size of the oral sucker in relation to the increases in length of the body of a series of females [Figs. 2 (F.), 3 (F.), 4 (F.), 5 (F.), 7 (F.) and 8 (F.) [, with a comparable series of developmental stages of males [Figs. 3 (M.), 4 (M.), 7 (M.), 9 (M.), 10 (M.), 14 (M.)] (see also Table IV). In this series of females the body length increases from 0.27 mm. to 1.85 mm., while the diameter of the oral sucker only increases from 0.05 to 0.092 mm. On the other hand, in the series of males the body length increases from 0.3 mm. to 3.1 mm., and the diameter of the oral sucker from 0.074 mm. to 0.227 mm. Katsurada records in fully developed males (Table III) measurements of the oral sucker ranging from 0.456 mm. to 0.604 mm. There is very little, if any, increase in size in the oral sucker of the female beyond the stage of 8 F.

The ratio in size of the oral sucker and the ventral sucker shows an interesting fluctuation in the course of the development, both of the male and the female. In the cercaria the oral sucker is between three and four times the size of the ventral sucker. At the stage in development, represented by Figs. 4 (F.) and 4 (M.) the suckers are very nearly equal in size. From this time on (see Table IV), the ratio is reversed, and the ventral sucker is constantly larger than the oral sucker.

The most striking change in the development of the oral sucker is the increase in the size of the mouth and buccal cavity. Even at the stage of Fig. 1 (F. or M.) the width of the mouth has already increased greatly beyond the condition in the cercaria (Text figs. 1 and 2), and the buccal cavity is beginning to assume the characteristic funnel shape. As development proceeds, the mouth increases rapidly in size [cf. Figs. 2 (M.), 3 (M.), 4 (M.), or Figs. 2 (F.), 3 (F.), 4 (F.)], coming to occupy most of the ventral surface of the oral sucker. The buccal cavity soon assumes the condition approximating the adult [Text fig. 3 and Fig. 8 (F.)]. It can be seen, by comparing the oral suckers at the stages of these figures with the condition in the cercaria (Text fig. 2), that the asymmetrical condition of the oral sucker which is apparent in the cercaria persists in later stages. In Text fig. 3, for example, it can be seen that the distance from the anterior lip of the oral sucker to the passage into the esophagus is longer than the distance from the posterior lip to this point, and that consequently the same relation is true in the buccal cavity.

6. *Changes in the digestive system.*

The digestive system in the cercaria of *S. japonicum* (Text figs. 1 and 2) is not functional, and is in a very rudimentary stage of development. This is especially indicated by the fact that the intestinal region, which attains such an extensive development in the adult [Figs. 11 (F.) and 18 (M.)], is represented in the cercaria only by a heart-shaped sac (Text fig. 1, *ds*). The remarkable changes which this system must pass through to attain the adult condition may be divided into three periods, i.e., (1) from the condition in the cercaria to the beginning of functional activity, (2) from the beginning of functional activity to the union of the intestinal ceca, (3) from the union of the ceca until completion of development. Of the first period, we know but little, altho Miyagawa's (1916) account of the development of this system indicates that functional activity is attained very soon after penetration (see literature section). The second period is characterized by the growth of the intestinal ceca. During this period, as indicated by Miyagawa's (1916) account and my own earlier stages, there is intense functional activity which is indicated by the very great distension of the intestine with food material [Figs. 3 (F.) and 3 (M.)]. The third period is characterized by a very rapid extension of the intestine, to keep pace with the growth of the body.

My earliest stage [Fig. 1 (M. or F.)] shows a great advance in the development of the digestive system over the cercaria, since the intestinal ceca are well developed and distended with brownish food material, indicating functional activity. There is, therefore, a big gap in my material, which includes the early stages of organization of the digestive system in preparation for functioning. Miyagawa's account (see section on literature) partly fills this gap. This organization must come very early, since the young worm needs an abundant supply of food, as soon as it has entered the final host, to prepare itself for the period of rapid growth. In my earliest stage [Fig. 1 (M. or F.)] the esophagus is well developed and already surrounded by the characteristic glandular area. This esophageal gland is made up of closely packed unicellular glands, which open separately into the esophagus. The only noticeable changes in the esophagus, as development proceeds, are increases in its diameter and length, corresponding to increases in size of the pre-acetabular region.

The development of the intestinal ceca up to the time of union is shown in Figs. 1 (M. or F.), 2 (M.), 2 (F.) and 3 (M.). These figures show an elongation of the intestinal ceca, corresponding to the increase of the post-acetabular region, and an approach of the ends of the ceca which fore-shadows their union. The most characteristic thing during this period is the great distension of the intestine with brownish food material, which makes its bulk very large in proportion to the size of the post-acetabular region of the body. The union of the intestinal ceca comes, when the developing worms have reached a length of about 0.3 mm. to 0.4 mm. [Figs. 3(F.) and 4 (M.)]. For a considerable time after the union of the ceca there is still noticeable a considerable distension of the intestine with food material [Figs. 5 (F.), 5 (M.) and 7 (M.)]. As the length of the post-acetabular region of the body increases, and the intestine becomes longer, it is less distended with food material, and assumes more the narrow irregular appearance of the fully developed condition [Figs. 5 (F.), 7 (F.), 8 (M.) and 9 (M.)]. In the later stages of development the intestine of the male becomes wider than that of the female. From cross-sections, however, it can be seen that this difference is compensated for, in the female, by a greater thickness of the intestine. These differences are of course, correlated with the differences in thickness of the body in the two sexes.

The relative length of the divided and the united regions of the intestine, at various stages, can be seen from Table IV. At the time of union of the intestinal ceca, two points are definitely established, i.e., (1) the point of bifurcation of the intestinal ceca which remains throughout all the further stages of development just in front of the base of the ventral sucker, and (2) the posterior tip of the intestine which remains always very close to the posterior tip of the body. As the body length increases, these two points remain constant and there is an increase in length of both the divided and the united portion of the intestine, to keep pace with the increase in the body length.

Soon after the union of the intestinal ceca a distinct sexual difference can be noted. In the adult female, the area just in front of the intestinal ceca is much wider than in the male, since it contains the ovary and other parts of the reproductive system. An examination of the figures of the early stages of the female [Figs. 3 (F.), 4 (F.), 5 (F.), 6 (F.)] shows that this area is already apparent at an early stage of development, and contrasts with the condition of this region in the male [Figs. 4 (M.), 7 (M.), 8 (M.)]. This difference is one of the points of sexual dimorphism which makes early discrimination of the sexes possible.

7. *Development of the reproductive organs.*

No attempt was made to trace the histogenesis of the reproductive organs. I will note, however, certain of the important stages in development. It is only late in development that the reproductive organs themselves become clearly differentiated. In the male it was difficult to make out the outlines of the reproductive organs in the early stages. In a male, 2.5 mm. in length, taken from a mouse nineteen days after infection, the outlines of the seminal vesicle and the testes were clear. The testes were almost round, and composed of dense nuclei, the spermatogonia, with considerable space between. The testes at this stage had an embryonic appearance, and there was no trace of spermatogenesis. The length of the total testes group of seven, was 0.175 mm., and the testes had a diameter ranging from 0.023 to 0.032 mm. The testes had distinct spaces between them. A

TABLE IV.

*Measurements of a series of development stages of Schistosoma japonicum in mm.**

Individual Characteristics.	1 (F. or M.).	2 (F.).	3 (F.).	4 (F.).	5 (F.).	6 (F.).	7 (F.).	8 (F.).	10 (F.).	11 (F.).	2 (M.).	3 (M.).
Length	0.24	0.27	0.325	0.48	0.81	0.91	1.1	1.85	4.2	8.2	0.3	0.3
Width at ventral sucker	0.1	0.1	0.1	0.125	0.12	0.11	0.1	—	—	0.1	0.11	0.115
Width at middle of pre-acetabular region	0.08	0.085	0.085	0.075	0.1	0.09	0.075	—	—	—	0.095	0.1
Width at middle of post-acetabular region	0.1	0.1	0.115	0.12	0.14	0.12	0.12	0.1	0.15	—	0.095	0.12
Length of pre-acetabular region	0.15	0.13	0.14	0.18	0.23	0.2	0.12	0.2	0.25	0.45	0.16	0.15
Percentage of total length taken up by pre-acetabular region	62.0	48.0	43.0	37.5	28.0	22.0	10.0	11.0	6.0	5.0	53.0	50.0
Ratio of length to width	—	—	33 : 10	—	20 : 3	—	12 : 1	18 : 1	28 : 1	—	30 : 11	—
Length of oral sucker	0.063	0.059	0.069	0.067	0.071	—	0.057	0.075	—	0.1	—	0.069
Width of oral sucker	0.055	0.05	0.05	0.063	0.084	—	0.092	—	—	—	—	0.074
Length of ventral sucker	0.034	0.042	0.053	0.067	0.067	—	0.067	0.099	—	0.1	—	0.035
Width of ventral sucker	0.038	0.059	0.061	0.068	0.084	—	0.097	—	—	—	—	0.059
Length of bifurcated part of intestine	0.101	0.126	0.153	0.168	0.378	—	0.609	—	—	—	—	0.161
Length of united part of intestine	—	—	0.042	0.095	0.189	—	0.174	—	—	—	—	—
Width of intestine	0.025	0.025	0.044	0.067	0.042	—	0.021	—	—	—	—	0.032

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* The numbers given to the individuals in Table IV correspond to the numbers given to the figures in Plates I. to IV.

TABLE IV (*continued*).

Individual Characteristics.	4 (M.).	5 (M.).	7 (M.).	8 (M.).	9 (M.).	10 (M.).	11 (M.).	13 (M.).	14 (M.).	15 (M.).	17 (M.).	18 (M.).
Length	0.44	0.56	0.8	1.07	1.5	1.8	1.7	2.2	3.1	3.8	5.6	8.1
Width at ventral sucker	0.13	0.15	0.16	0.21	0.25	0.24	0.25	—	—	—	—	0.2
Width at middle of pre-acetabular region	0.11	0.12	0.12	0.16	0.22	0.18	—	—	—	—	—	—
Width at middle of post-acetabular region	0.14	0.15	0.14	0.19	0.22	0.28	—	—	—	—	—	—
Length of pre-acetabular region	0.21	0.26	0.25	0.34	0.47	0.4	0.5	0.5	0.65	0.6	0.85	0.7
Percentage of total length taken up by pre-acetabular region	47.0	40.0	31.0	32.0	31.0	22.0	29.0	23.0	21.0	16.0	18.0	9.0
Ratio of length to width	44 : 13	—	5 : 1	5 : 1	6 : 1	—	—	—	9 : 1	—	16 : 1	—
Length of oral sucker	0.088	—	0.105	—	0.202	0.168	0.197	—	0.179	0.252	—	0.3
Width of oral sucker	0.103	—	0.128	—	0.17	0.176	—	—	0.227	—	—	—
Length of ventral sucker	0.082	—	0.122	—	0.208	0.242	0.210	—	0.273	0.302	—	0.3
Width of ventral sucker	0.095	—	0.128	—	0.21	0.252	—	—	0.294	—	—	—
Length of bifurcated part of intestine	0.179	—	0.357	—	0.777	1.144	0.79	—	1.59	1.69	—	—
Length of united part of intestine	0.08	—	0.168	—	0.225	0.426	0.336	—	0.84	1.4	—	—
Width of intestine	0.042	—	0.057	—	0.021	0.063	—	—	—	—	—	—

more advanced stage in development was seen in a male, 3.7 mm. in length, taken from a mouse twenty-one days after infection. The testes in this form were denser and appeared to be in the process of spermatogenesis. This male was just a little smaller than the smallest male found in copulation. Probably, therefore, it represents the stage of the beginning of sexual maturity. The total length of the testes group in this form was 0.205 mm. The individual testes were compact bodies, somewhat rounded, but wider than long. The length of the individual testes was about 0.023 to 0.032 mm., and their width 0.042 to 0.048 mm. The anterior and posterior testes were almost round, and distinct spaces were visible between the individual testes.

The fully matured condition was represented by a male 8 mm. in length [Fig. 18 (M.)], taken from a mouse thirty days after infection. In this specimen the total length of the testes area was 0.532 mm. The dorso-ventral diameter of the middle five testes was greater than their length, while the anterior and posterior ones were practically spherical. The smallest testes in the group had a width of 0.095 mm. and a length of 0.067 mm., while the largest had a length of 0.076 mm. and a width of 0.105 mm. The diameter of the end ones was 0.095 mm. These testes were very compact, surrounded by a definite connective tissue sheath, and were in contact.

In males of about 1 to 2 mm., traces of the reproductive organs could be made out, but their outlines could not be clearly distinguished. In earlier stages I was unable, in the material studied, to find the *Anlage* of the male system.

In very early stages the *Anlage* of the ovary can be distinguished in the widened area in front of the place of union of the intestinal ceca. The smallest form in which I was able to distinguish any of the other organs was a specimen 1.44 mm. in length, taken from the liver of a mouse, eighteen days after exposure to infection. At this stage the ovary had a length of 0.095 mm. and a width of 0.030 mm. Just in front of the ovary the position of Mehlis' gland could be distinguished. The uterus was very clearly defined and could be traced, as a straight tube, in the region between the intestinal ceca, from just in front of Mehlis' gland to the genital pore. Parts of both the vitelline duct and oviduct could be distinguished, and the position of the vitellaria was indicated by denser areas in the posterior end of the body. At this early stage, therefore, all parts of the reproductive system are differentiated.

In another specimen, 3.2 mm. in length, which was much coiled, the ovary had a length of 0.171 mm. and a width of 0.042 mm. The outlines of the lobes of the vitellaria could be clearly distinguished along each side of the body for the whole length of the post-ovarian region, but they did not have the characteristic brownish color which comes with functional activity. The outlines of the other organs were clearly defined. This form evidently represents a stage, just before sexual maturity was attained. In a fully matured specimen [Fig. 11 (F.)] the ovary had a length of 0.4 mm. and a width of 0.15 mm., the vitellaria were very dense, and had a brownish color. There were in this specimen about 50 eggs in the uterus.

VII. SUMMARY.

1. The contrast is very striking between the cercaria of *S. japonicum,* in which the structure of the body is greatly modified by adaptive larval characters for locomotion and penetration, and the adult in which the sexes are separate and there is an extreme sexual dimorphism.

2. Sexual dimorphism has never been demonstrated in the cercariae of the human schistosomes, although the finding of a size dimorphism in a closely related species of schistosome cercaria suggests that undetected differences may be present.

3. The finding by Japanese workers, that when an experimental host is infected with cercariae of *S. japonicum* from a single snail, all the resulting individuals are in the great majority of cases of the same sex, and my own findings that in the schistosome cercaria from Douglas Lake, in fifteen infected snails studied, all the cercariae from a single snail were of the same size type, suggests the theory that sex in the schistosomes is differentiated in the miracidium stage, and that all cercariae which develop from a single miracidium are of the same sex.

4. The work of Japanese investigators on the life history of *S. japonicum* has demonstrated that infection with this species is always by penetration of the cercaria through the skin or the oral or pharyngeal mucous membrane, and never by way of the digestive tract, and that the cercariae can penetrate easily at any point through wet skin, although the most frequently enter by way of the hair follicles.

5. The course by which the larval blood fluke reaches the blood vessels of the liver has been the subject of considerable controversy. Soon after exposure to infection, the larvae will be found in the epidermis, dermis and subcuticula, and later most of them enter the

small blood vessels and lymph spaces. They are carried to the heart, either by the venous system or by the lymph vessels, although the former seems to be the most frequent course, since most of those which get into the lymph system seem to die in the lymph glands. From the heart they are carried to the lungs. From the blood vessels of the lungs, two courses have been traced. The first course is through the capillaries of the lungs back to the heart, from the heart by the arterial system, and through the capillaries to the hepatic portal system. Those larvae which follow the second course which is probably by far the most frequent, leave the blood vessels in the lungs, penetrate through the lung tissue, enter the peritoneal cavity by way of the connective tissue of the posterior mediastium and to a lesser extent of the anterior mediastinum, following with preference the course of the large blood vessels and nerve trunks and finally penetrate into the liver, where they enter the hepatic portal system.

6. Soon after penetration throuh the skin, the tail of the cercaria is lost and the cephalic and head glands degenerate. There is very little increase in size, until the larvae reach the liver, altho the digestive system becomes organized for functioning, and the oral sucker begins to assume its true character.

7. In the earliest stage found in the liver, it is impossible to distinguish the sexes, but at the stage between 0.3 mm. to 0.4 mm. the males show distinctly larger suckers, and a broader body than the females, and the females have a distinctly broader area in front of the union of the intestinal ceca, where the ovary later develops.

8. To attain the largest size recorded for this species the cercaria must increase in length over 100 times. The most rapid period of growth is from the time of reaching the liver to sexual maturity, when the worms may increase their length in less than 20 days by 20 to 30 times. Growth continues after sexual maturity, but is much slower.

9. The finding of larvae as small as 0.3 mm. in the liver, up to eighteen days after infection, can be explained by postulating that the worms grow but little in size, until they reach the liver, and that there is a variation in time for the migration course.

10. The increases in size are almost entirely in the post-acetabular region. As growth goes on, there is a very great increase in the ratio of length to width, which is much greater in the female than in the male. The body of the female soon becomes much narrower than in males of the same age, and becomes round in cross-section, while in the male the post-acetabular region gradually becomes flattened, and the sides grow up to form the gynaecophoric canal.

11. The size of the suckers increases greatly in the course of development, but not in proportion to the increases in body size. In the male, this increase is much greater than in the female. In the cercaria the ventral sucker is only about one third of the diameter of the oral sucker. In an early stage of development, the suckers become about equal in size, and in later stages the ventral sucker is constantly larger than the oral sucker. The ventral sucker with increase in size becomes thinner, and juts out from the body, until in later stages it is saucer-shaped and has a distinct peduncle. The greatest change in the oral sucker is the great increase in the size of the mouth and buccal cavity.

12. From the very rudimentary condition of the cercaria the digestive system soon becomes horseshoe shaped, and very much distended with food material. The ends of the intestinal ceca unite at a stage of about 0.4 mm. in length. The divided and united portions both extend greatly, to keep pace with the increased length of the body. In the female the position of the point of union of the intestinal ceca and the greater width of the area in front of this point as compared with the male is influenced by the development here of the ovary.

13. Differentiation of the reproductive organs comes late. Although at an early stage, the *Anlage* of the ovary can be distinguished, it is not until the female attains a length of about 1.5 mm., that the outlines of the ducts and other organs can be clearly made out. In the male it was only in specimens over 2 mm. in length that the outlines of the testes could be clearly distinguished.

EXPLANATION OF PLATES.

All figures were drawn with a camera lucida and represent different stages in the development of *S. japonicum,* in experimentally infected mice. The numbers of the figures correspond to the numbers given to the individuals in Table IV.

Abbreviations used:

e, eggs; *eg,* esophageal gland; *es,* esophagus; *gf,* fold of gynaecophoric canal; *i,* intestine; *is,* intestinal space in female, where ovary develops; *n,* central nerve mass; *os,* oral sucker; *ov,* ovary; tt, testes; *vit,* vitellaria; *vs,* ventral sucker.

PLATE I.

FIG. 1 (F. or M.) Very immature specimen from liver of mouse, ten days after infection; length 0.24 mm., sex not certain, ventral view.

FIGS. 2 (F.), 3 (F.) AND 4 (F.). Very immature females from mouse's liver, twelve days after infection, ventral view.

Figs. 2 (M.) and 3 (M.). Very immature males from liver of mouse, twelve days after infection, ventral view.

Fig. 4 (M.). Very immature male from liver of mouse, sixteen days after infection, ventral view.

Plate II.

Figs. 5 (M.) and 7 (M.). Immature males from liver of a mouse, fourteen days after infection, ventral view.

Figs. 8 (M) and 9 (M.). Immature males from the liver of a mouse, sixteen days after infection, ventral view.

Fig. 5 (F.). Immature female from liver of a mouse, fourteen days after infection, ventral view.

Fig. 6 (F.). Immature female from liver of a mouse, sixteen days after infection, ventral view.

Fig. 7 (F.) Immature female from liver of a mouse, eighteen days after infection, ventral view.

Fig. 8 (F.). Immature female from liver of a mouse, nineteen days after infection, side view.

Plate III.

Fig. 9 (F.). Immature female from liver of a mouse, nineteen days after infection, side view.

Fig. 10 (M.). Immature male from liver of a mouse, twenty-one days after infection, ventral view.

Fig. 11 (M.). Immature male from liver of a mouse, nineteen days after infection, side view.

Fig. 13 (M.). Immature male from liver of a mouse, eighteen days after infection, side view.

Fig. 14 (M.). Immature male from liver of a mouse, nineteen days after infection, ventral view.

Figs. 17 (M.) and 10 (F.). Male and female in copulation from liver of infected mouse, twenty-one days after infection.

Plate IV.

Fig. 18 (M.). Sexually mature male from liver of mouse, thirty days after infection.

Fig. 11 (F.). Sexually mature female from liver of mouse, thirty days after infection.

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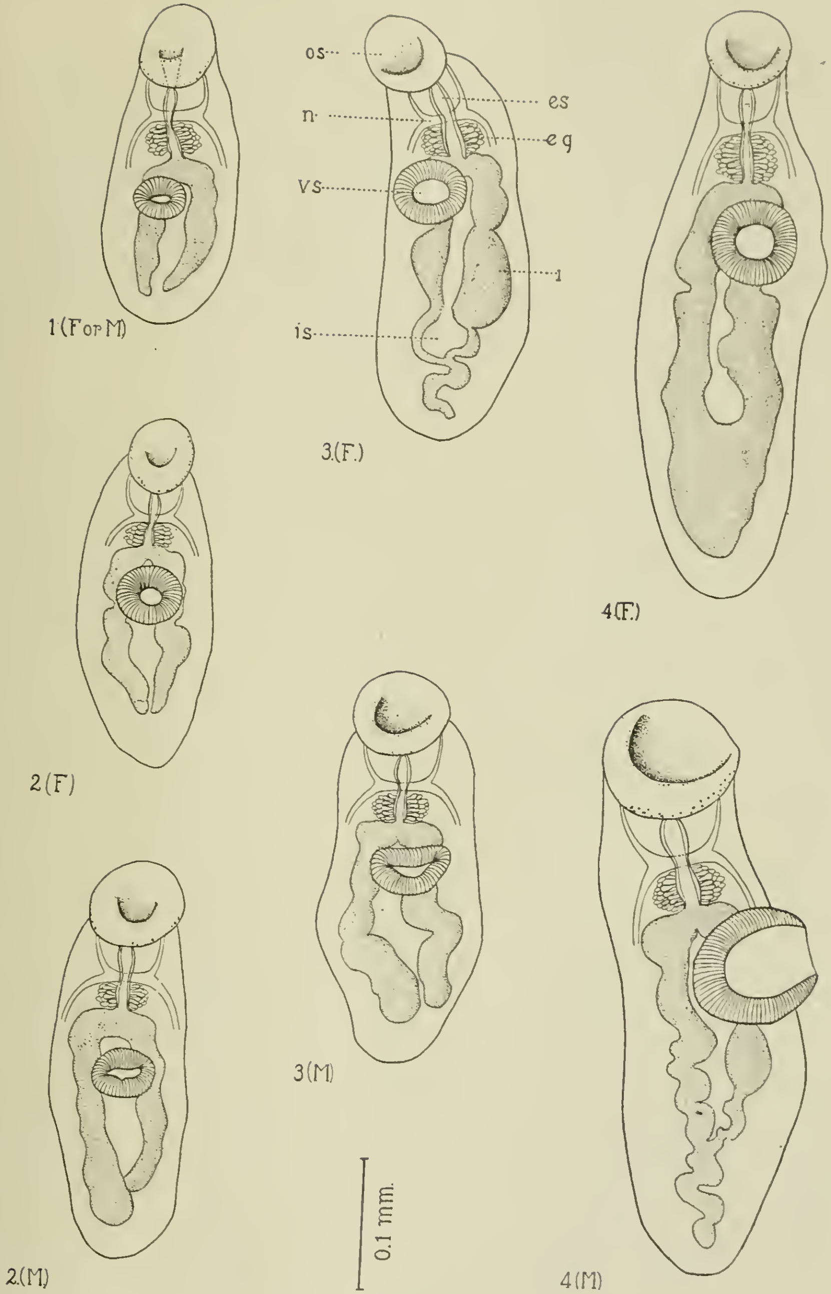
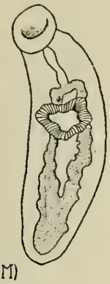


1 (F or M)
3 (F.)
4 (F.)
2 (F)
3 (M)
2. (M)
4 (M)

PLATE I.



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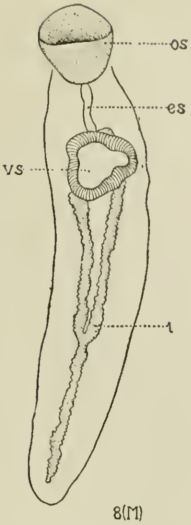

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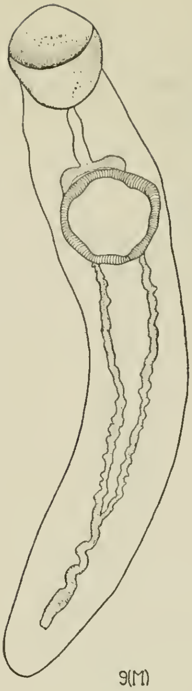
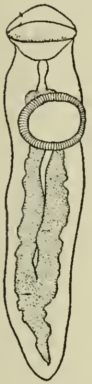


9(M)



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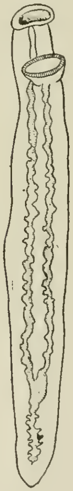

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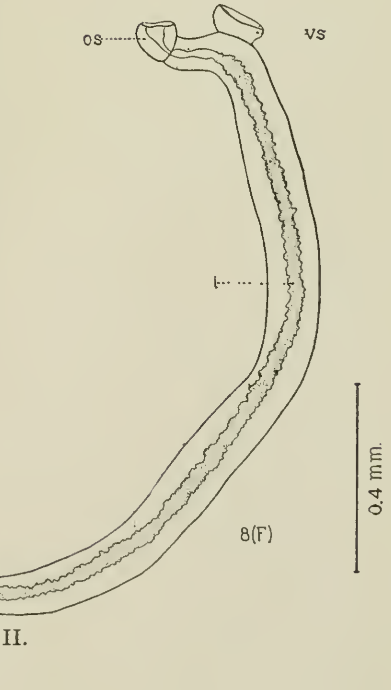

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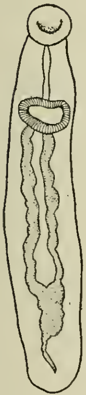

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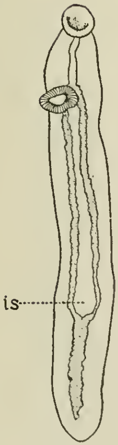
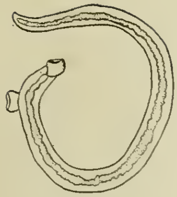
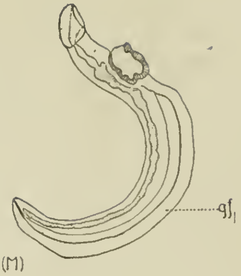


6(F)

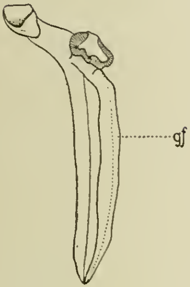
PLATE II.



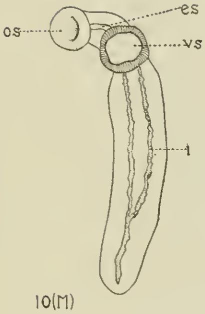
9(F)



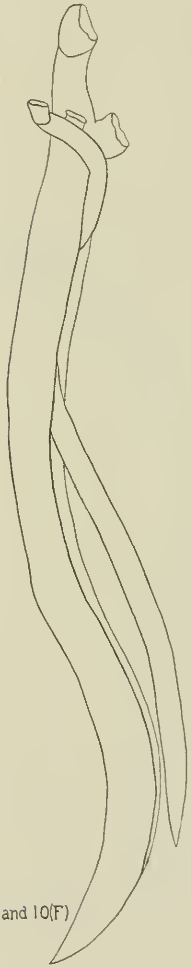
13(M)



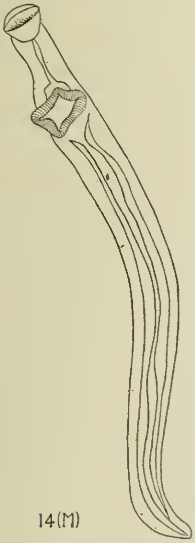
11(M)



10(M)



17(M) and 10(F)



14(M)

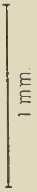


Plate III.

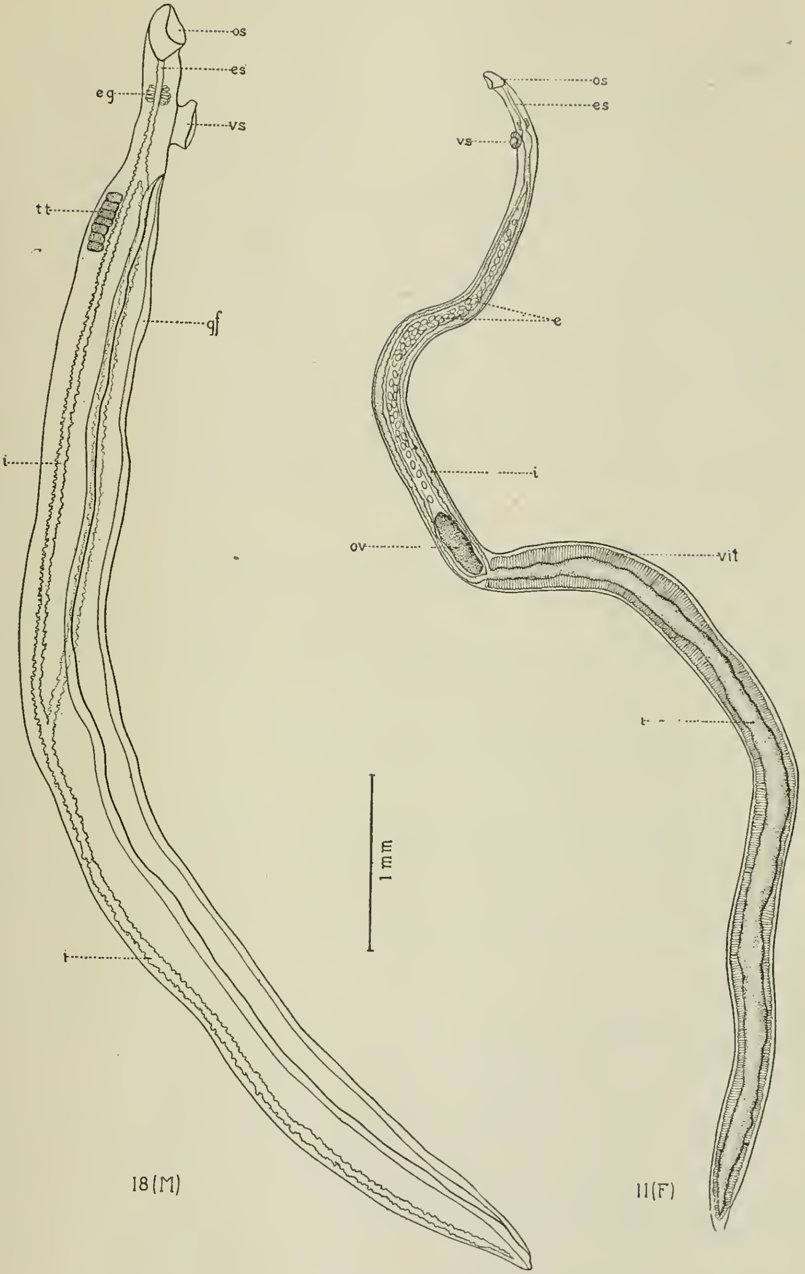


PLATE IV.

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THE ACTION OF LIGHT ON THE LEUCOCYTE COUNT.

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It has been generally supposed that light produces some effect on the blood, but the experiments which have been done to test this point are many of them unsatisfactory, and the results reported are inconclusive.

In speaking of light one includes, not only the visible spectrum, from 400 to 760 $\mu\mu$, but also the ultra-violet region from 100 to 400 $\mu\mu$, and the infra-red of wave-length longer than the visible. The ultra-violet should be divided into two parts; the near ultra-violet, i.e., the wave-lengths just below the visible, from 300 to 400 $\mu\mu$, and the far ultra- violet, from 100 to 300 $\mu\mu$. The physiological effects produced by these two regions are strikingly different. In previous work practically no attempt has been made to study different wave-lengths of light separately. Experiments have usually been made with sunlight which extends only to 291 $\mu\mu$ in the ultra-violet, and includes, therefore, near ultra-violet and visible light and a large amount of infra-red. Owing to the absorption of water vapour in the atmosphere, very little infra-red of wave-length longer than 400 $\mu\mu$ is transmitted.

The effect produced by light on the erythrocyte count has been found in general to be negative. Long-continued absence of light certainly produces no marked change in the number of red cells. Blessing (1), who acted as physician to Nansen during his expedition in the Fram, published a report in 1898, showing that members of the party exhibited no evidence of anemia during the trip. Similar results were reported for animals by Grober and Sempell (2) in 1919. They examined horses that had worked for years in coal mines, and found no anemia in any case where a satisfactory nutritive condition existed. Borissow (3) kept rabbits and dogs in the light and the dark and reported negative results on the erythrocyte count.

A number of investigators, however, have performed similar experiments with positive results. Graffenberger (4) in 1893 reported that rabbits showed an increase in the percentage of hemoglobin in the light and a decrease in the dark. This result was confirmed by Marti (5) in 1897. He found that rats kept in the dark showed a decrease in the red count and in the percentage of hemoglobin, while those exposed to sunlight, in the day, and an arc light at night, had an increased count. Orum (6) reports similar results, although he introduces a new factor in that he lays emphasis upon changes in blood volume caused by light. Exposure to darkness or to red light causes an initial decrease in the red count and the percentage of hemoglobin, but after a month or two the count and percentage of hemoglobin are increased, owing to a marked concentration of the blood. In white light, and especially in blue light, there is, on the contrary, a condition of plethora, together with an increase in the total hemoglobin. It would seem to be highly desirable to have these experiments repeated with more modern methods of determining the blood volume. If the effects on volume of long continued exposures to red and violet lights are as striking as described by Orum, they would indicate an actual decrease in red corpuscles in the dark (or red), and an increase in the white (or violet), compensated more or less in each case by a diminished or an increased volume of the blood.

All the positive results reported agree and give considerable evidence for believing that there is a decrease in the red count and in the percentage of hemoglobin in the dark, and an increase in the light. These changes, however, are not permanent and the blood count later returns to normal.

The leucocytes show a greater and more definite response to short exposures to light than the erythrocytes. Polito (7) exposed rabbits to direct sunlight for periods of time ranging from fifteen minutes to an hour. The length of exposure made practically no difference, and he reports an increase in the leucocyte count immediately after exposure, amounting to from 2,000 to 5,000 in the total count, with most of the increase in the mononuclear cells. After an hour the blood returned to normal, and unfortunately was not studied further. These animals were exposed to direct sunlight at a temperature of 38° to 42° C., so that the light effect was seriously complicated by a possible heat effect. Murphy (8) has shown that five minutes' exposure of an animal to dry heat, at 55° to 65° C., will produce a rise in lymphocytes of 100 to 200 per cent. over the normal count.

Aschenheim (9) reported that an exposure of infants to direct sunlight, for an hour, usually brought about an increase in the number of leucocytes, the neutrophiles being relatively diminished and the lymphocytes relatively increased.

In 1919 a paper was published by Taylor (10) on the effect of exposure to the sun on the circulating lymphocytes in man. Thirty-eight people were examined before and after a summer at Woods Hole, Massachusetts. In twenty-five of the thirty-eight there was an appreciable increase, both percentage and absolute, in the number of circulating lymphocytes. The differential counts showed an average increase from 27.4 to 35.6 per cent. Eight subjects showed a decrease and five no appreciable change. Of these six failed to tan and three were very dark to begin with. It seems hardly justifiable to attribute this increase solely to ultra-violet light when other agencies were active, such as change in climate and mode of life

It is, however, interesting in this connection to find that the blood counts made by Chamberlain, Vedder, Phalen, Wickline and others, on Americans in the Philippines and on native Philippinos, show an increase in the percentage of lymphocytes and a decrease in polymorphonuclears, as a result of residence in the tropics. Their results have been summarized briefly in the following table, from Chamberlain and Vedder (11) (see Table I).

TABLE I.

Observer.	Race.	Number observed.	Poly-morpho-nuclears.	Small lympho-cytes.	Large lympho-cytes.	Total count.
Wickline (12).....	Americans, 18 months' service	104	54.9	33.4	6.1	6,831
Guerrera (13) and Sevilla.........	Philippinos	129	51.6	34.5	4.1	—
Phalen (14).......	Americans, more than one year's service	115	58.7	32.6	4.6	—
Chamberlain (15) .	Igorots	40	46.9	37.2	5.7	—
Chamberlain (11) and Vedder.....	Americans, 14 months' service	72	56.8	31.7	6.9	7,304
Chamberlain (11) and Vedder.....	Philippinos	50	52.2	29.9	6.6	9,248
Simon (16).......	Normal count, temperate climates	—	60 to 70	20 to 30	1 to 6	5,000 to 10,000

Wickline (12) has shown that these low polymorphonuclear and high lymphocyte counts, for Americans living in the Philippines, developed gradually. He made counts on 104 soldiers, on arrival and 3, 12, and 19 months later. After 3 months the mononuclear cells averaged 31.4 per cent., after 12 months 34.4 per cent., and after 19 months 39.5 per cent. The small mononuclears, which undoubtedly correspond to the small lymphocytes, give a percentage of 21.8, 26.6, and 33.4 at the three examinations. During this change the total count stayed nearly constant, being 6,943 at the preliminary examination, and 6,699, 6,624, and 6,831 after 3, 12, and 19 months, respectively. How much of this effect is due to the action of tropical light is impossible to say. There has been a very prevalent idea that the solar spectrum extends further into the ultra-violet in the tropics. This is not true. The solar spectrum has been photographed at Manila, by means of a Rowland grating, by Freer (17) and was found to extend to 291 $\mu\mu$. Miethe and Lehman (18) made measurements at Assuan, Berlin, Zermatt, Görnergrat and Monte Rosa and found the limit of the spectrum always the same, 291.55 $\mu\mu$ to 291.21 $\mu\mu$. Neither latitude nor altitude thus makes any appreciable difference in the extent of the solar spectrum in the ultra-violet. However, light in the tropics has greater intensity than in temperate zones, so that in this way there may be a greater exposure in the tropics to ultra-violet light, as well as to visible light and heat. Most of the effects produced on soldiers in the tropics, such as increased pulse rate, respiration, and body temperature, can also be produced by moist heat. (Phalen 14.) Whether the blood changes are due to this cause, or whether they result from an increased exposure to ultra-violet light, is at present impossible to say.

In general, however, one can conclude from former work that exposure to sunlight results in an increase in lymphocytes. The experiments have all been complicated by the action of heat and are suggestive rather than conclusive.

In the experiments reported here an attempt was made to study separately the effects of different regions of visible and ultra-violet light, using short exposures.

METHOD.

Rabbits were exposed to the light of an iron arc for one hour, at a distance of ten inches. At this distance there was no heat effect, the temperature remaining below 25° C. The rabbits were enclosed in a box, with only the head exposed, and the eyes were covered during exposure, so that only the ears were radiated. The hair was clipped from the ears before radiation, so that the skin was well exposed. Blood counts, both total and differential, were made before and after exposure, and the animals were then returned to a basement room, very dimly lighted by diffuse light, in which all animals had been kept some weeks or months before being used. The blood was counted daily until a normal condition seemed to be reestablished. This usually occurred in about three weeks.

The iron arc gives a brilliant light which, when examined spectroscopically, is so rich in lines that it almost amounts to a continuous light source. It reaches to about 238 $\mu\mu$, and is strong throughout the ultra-violet. No quantitative work has been done on the relative intensity of the iron arc throughout the spectrum. Pflüger (19) investigated the energy distribution in the spark spectrum of iron, with a bolometer, and found the greatest energy in the ultra-violet. The distribution in the arc is very different from that in the spark, but it can be assumed that the ultra-violet region is at least as intense as the visible, and probably of greater intensity. In Fig. 1 the distribution of energy in the solar spectrum is given, showing that in the sunlight there is a great exposure to visible light and a very weak exposure to ultra-violet, while the reverse is true of the iron arc.

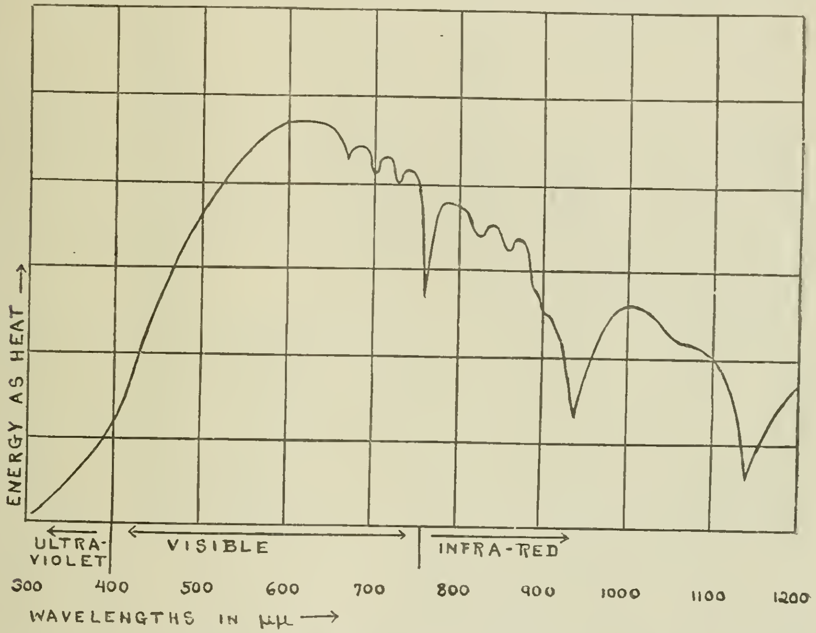

FIG. 1. Distribution of energy in the solar spectrum (Langley). Ordinates —energy as heat, units arbitrary. Abscissae—wave-lengths in $\mu\mu$.

In order to investigate separate regions of light a number of different filters were used. The absorption of these is well known, but the particular filters were tested by photographing the transmitted light with a meter focus concave grating spectrograph. The transmission of these filters is given in Table II.

TABLE II.

Source.	Transmission ($\mu\mu$).
Iron arc unscreened	238 →
Iron arc through glass	320 →
Iron arc through ultra-violet glass*	330 → 390
Iron arc through picric acid	450 → 750
Iron arc through red glass	650 →

It is impossible to isolate the ultra-violet below 300 $\mu\mu$ by means of a filter, as this region is strongly absorbed by all substances except those, such as quartz or fluorite, which also transmit the visible. By comparing the effect of the radiation with and without the plain glass screen it is possible to estimate which part of the effect is due to the far ultra-violet below 300 $\mu\mu$.

The absorption of blood is compared with the transmission of the filters in Fig. 2. It might be expected that the effect of light on the blood would be due to the absorption of light energy by some constituent of the blood, with resultant physical and chemical action. It can be seen that the region from 230 to 300 $\mu\mu$ is absorbed by the hemoglobin and also by the blood serum. The hemoglobin also absorbs the region from 300 to 450 $\mu\mu$, and has two absorption bands in the yellow, between 530 and 590 $\mu\mu$.

It is impossible to say anything very definite in regard to the absorption of the skin, although the experiments reviewed by Jesionek (20) show roughly that light of wave-length shorter than 300 $\mu\mu$ is absorbed by the epidermis, and that bloodless tissue will transmit some rays throughout the visible.

TECHNIQUE OF BLOOD COUNTING.

A word should be said about the technique of blood counting used in this work. Previous work, by the author, on rabbits had proved unsatisfactory, owing to large and inexplicable variations in the white count, although the red counts had been reasonably constant.

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* This ultra-violet glass was obtained from Dr. R. W. Wood. It absorbs the visible and transmits the near ultra-violet, just below the visible.

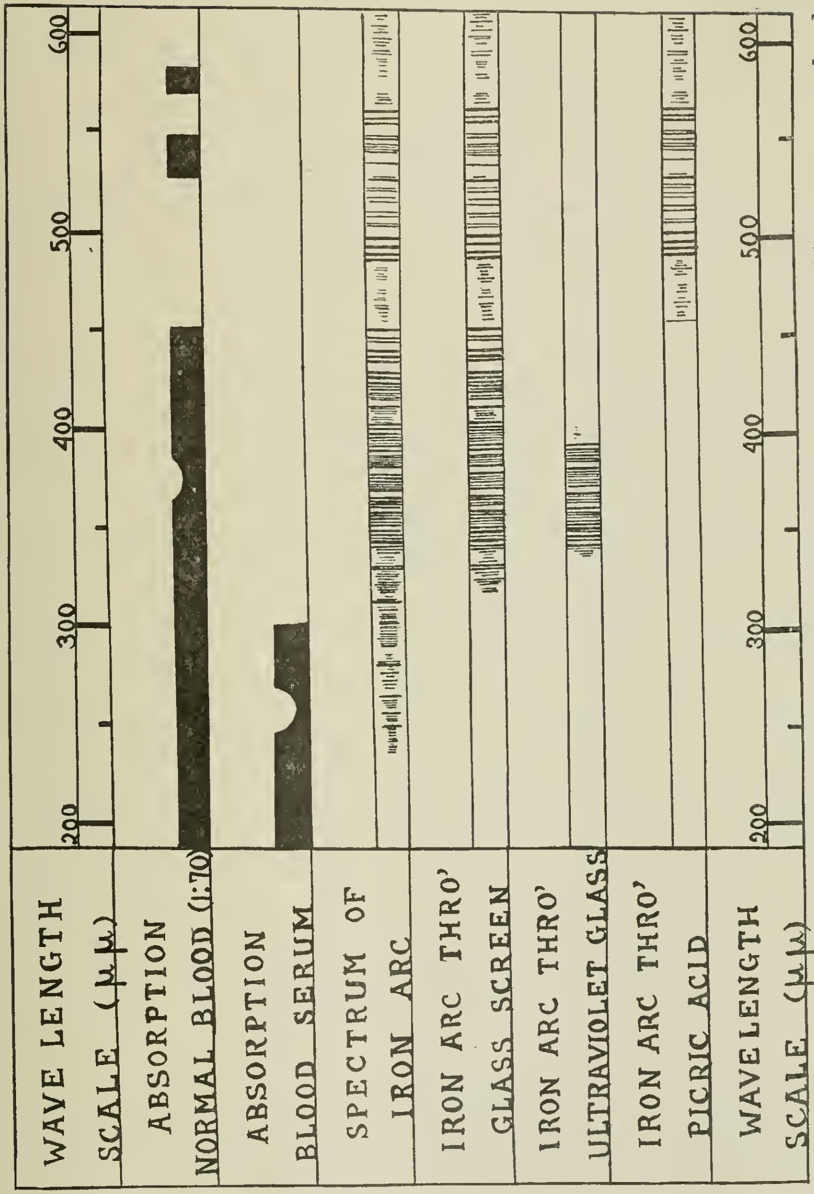

FIG. 2. Absorption spectra of blood and blood serum compared with emission spectra of iron arc unscreened and iron arc through filters.

Believing this to be due to different conditions of circulation in the ear when the counts were made, the leucocyte counts on the normal, untreated ear, and the ear when well flushed with hot water (45° C.) were compared. The results obtained are shown in Table III.

TABLE III.

Leucocyte Counts.

	Untreated.	Flushed once.	Flushed twice.
Rabbit I	16,640	11,700	—
Rabbit II	16,930	12,510	11,900

Apparently, on flushing the ear and getting a rapid circulation through it, the white count drops to a steady level, which is presumably that of the circulating blood. By following this technique very consistent counts were obtained. Some of the rabbits used gave very constant counts from day to day, when not subject to radiation. Others showed variations, and these responded to radiation with larger changes than the rabbits with constant counts, although the changes were of the same type.

In making the differential count the smears were stained with Jenner's stain and the following classification was followed; large mononuclears (cells in which the ratio of nucleus to cystoplasm is relatively small); lymphocytes (all cells, both large and small, in which this ratio is relatively large); polymorphonuclears (these are called pseudo-eosinophiles by Klieneberger and Carl (21), and correspond to the neutrophiles in man); eosinophiles, and mast cells. A normal differential count, as given by the non-radiated rabbits, is roughly as follows:

	Large mononuclears.	Lymphocytes.	Polymorphonuclears.	Eosinophiles.	Mast.
Per cent	1	64	30	1	4

RESULTS.

In the first experiments the total red and total white counts were followed for each animal, but no differentials were made. The erythrocyte count gave negative results. The leucocyte count, however, showed marked and consistent variations after exposure to light.

The red count was therefore abandoned, and total and differential white counts were made throughout the rest of the work.

Results of exposure to the unscreened iron arc (Figs. 3, 4 and 5).

Immediately after the exposure there is a sharp drop in the total count (Fig. 3). This is contrary to Polito's results (7), but the conditions of the experiment were also different from his. The differential (Fig. 4) shows this to be due entirely to a drop in the number of lymphocytes, so that it cannot be attributed to an increase in blood volume. By the next day the total count (Fig. 3) was approximately normal, and then rose steadily reaching a maximum on the fifth day. In the first experiments there was a sharp rise above normal on the second day. This was not marked in later experiments, due perhaps to the fact that the same animals were used repeatedly, and thereby

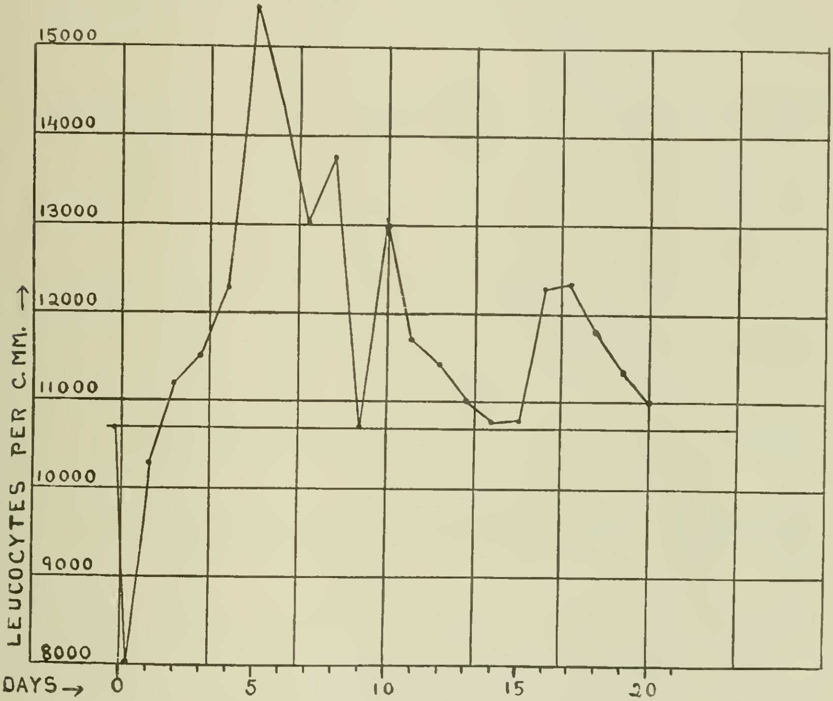

Fig. 3. Total leucocyte count, after exposure to unscreened iron arc (average of five rabbits). Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

lost their susceptibility somewhat. The differential curve (Fig. 4), however, shows a rise in lymphocytes on the second day after exposure, although it is much less marked than the rise on the fifth day. Comparing the total curve with the differential, the polymorphonuclears are seen to remain nearly steady throughout, though a little below normal, while the total curve and lymphocyte curve run almost parallel. The large maximum on the fifth day is followed by a return to normal on the ninth. This is followed by two small rises on the eleventh and sixteenth days. After three weeks the blood was apparently normal again, so that the experiments were not carried any further. The result of the exposure to the bare iron arc is an approximately normal polymorphonuclear count and, except for the initial drop immediately after exposure, a lymphocyte count above

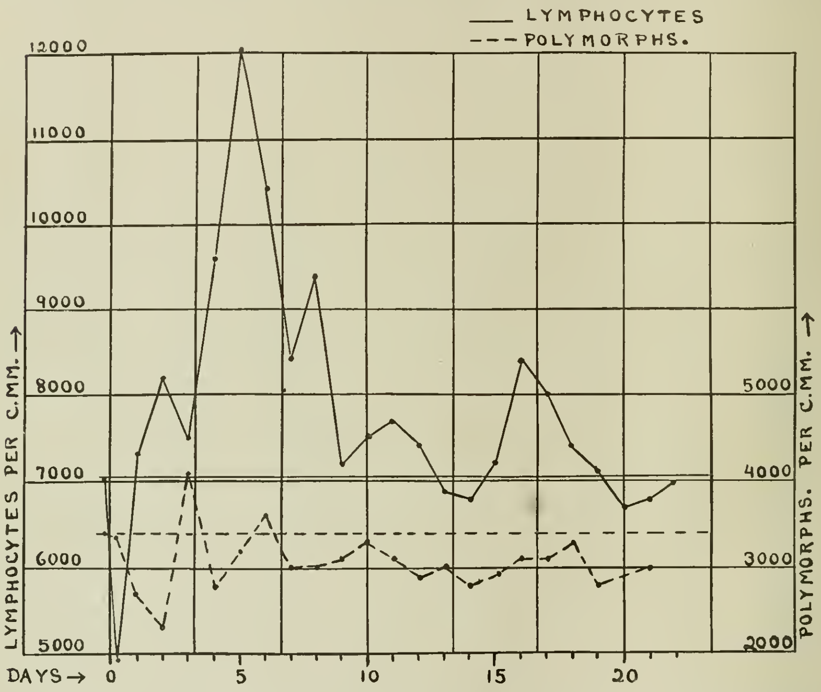

FIG. 4. Lymphocyte and polymorphonuclear counts, after exposure to unscreened iron arc (average of four rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure.
———————— lymphocytes — — — — — — — — polymorphonuclear leucocytes.

normal for three weeks. The maximum rise on the fifth day amounts to an increase of almost 100 per cent. over the original count.

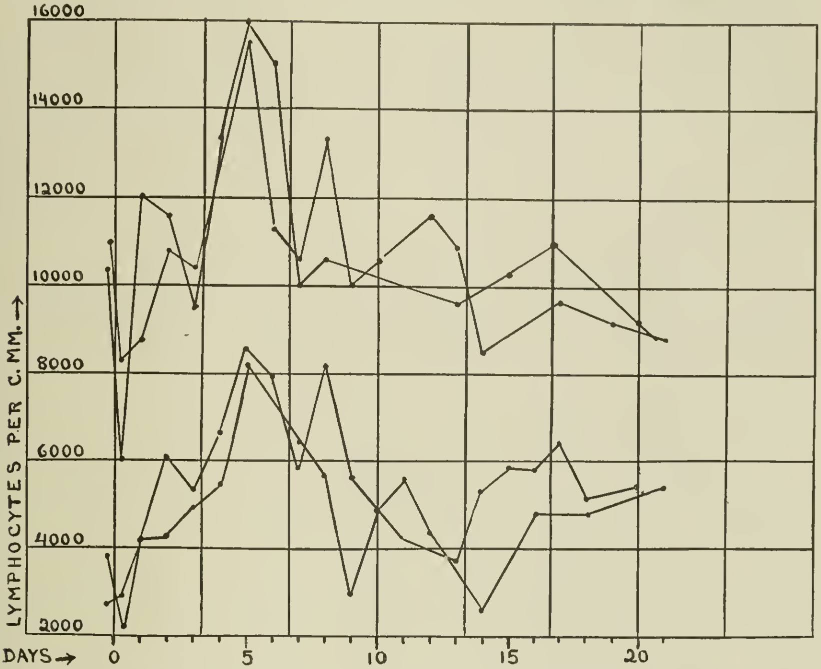

Fig. 5. Lymphocyte counts on four individual rabbits (same rabbits, averaged in Fig. 4), after exposure to unscreened iron arc. Ordinates—lymphocytes per c.mm. of blood. Abscissae—time in days after exposure.

In Fig. 5 the lymphocyte curves of four individual rabbits are plotted, in order to show how closely they agree with each other and with the average lymphocyte curve in Fig. 4.

Result of exposure to the iron arc through a glass screen

Fig. 6.

Only two animals were radiated through glass, but the results were sufficiently parallel to warrant drawing conclusions from them. The total leucocyte count and the absolute lymphocyte and polymorphonuclear counts are all plotted in Fig. 6. There is practically no change in the total count immediately after radiation, but the differential count shows a large decrease in lymphocytes, which is balanced by a corresponding increase in polymorphonuclears. The total count falls steadily until the third day, after which it fluctuates until the thirteenth day, although far below normal all the time. On the thirteenth and fourteenth days there is a marked drop, followed by a gradual rise to normal. After the initial rise and fall, immediately following the exposure, the polymorphonuclears stay approximately normal, except for a small rise on the seventh day. The decrease in total count is therefore due entirely to a fall in the number of lymphocytes. These stay far below normal for three weeks or more, and are reduced to one third of the original count on the thirteenth day after exposure.

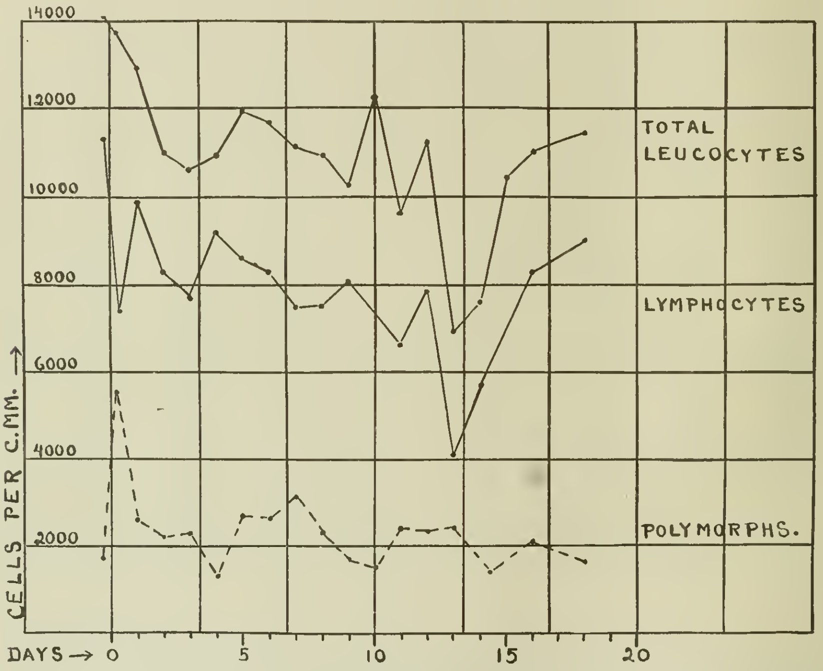

FIG. 6. Total leucocyte, and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through glass (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure——— ——— leucocytes and lymphocytes — — — — — — polymorphonuclear leucocytes.

The curves for radiation with the iron arc bare (Figs. 3 and 4), and with the iron arc through glass (Fig. 6), are strikingly different. Neither radiation produces any change in the number of polymorphonuclears after the first day, but both produce a very profound change in the number of lymphocytes. The lymphocyte count is greatly increased by radiation with the bare iron arc while it is decreased by radiation with the iron arc through glass. As the glass transmits everything above 320 μμ, one must conclude that the stimulating action is due to the region between 230 and 320 μμ; i.e., the far ultra-violet. It is probable that if this region could be isolated and used separately the increase would be much greater as the depressing action of the rest of the spectrum would not have to be counteracted.

RESULT OF EXPOSURE TO THE IRON ARC THROUGH ULTRA-VIOLET GLASS (TRANSMISSION 330 TO 390 μμ) (FIGS. 7, 8 AND 9).

In order to further analyze the action of light of wave-length longer than 320 μμ, the effect of special regions was investigated.

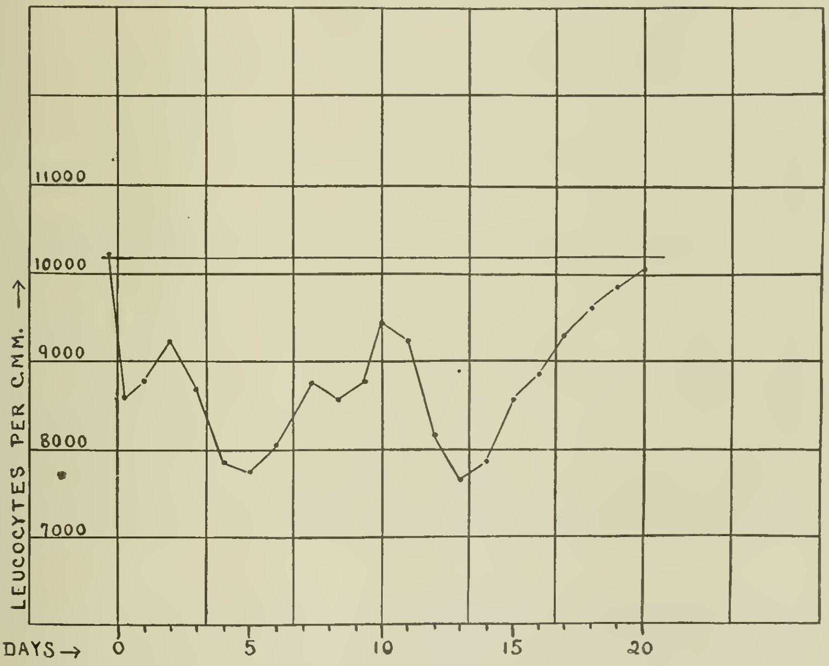

FIG. 7. Total leucocyte count, after exposure to iron arc through ultra-violet glass (average of five rabbits). Ordinates—leucocytes per c.mm. blood. Abscissae—time, in days, after exposure.

With Wood's ultra-violet glass the region between 330 and 390 μμ was tried. These experiments gave remarkably consistent results.

The total count (Fig. 7) shows a fall, followed by a rise on the second day. This is followed by a fall on the fifth day, a gradual rise on the tenth, another fall on the thirteenth and a gradual rise to normal. The differential (Fig. 8) shows that the initial fall is due entirely to lymphocytes, as the polymorphonuclears rise slightly. The rise on the second day is due to lymphocytes alone, but the two falls on the fifth and thirteenth days are due to both polymorphonuclears and lymphocytes. This region therefore causes a decrease in both polymorphonuclears and lymphocytes, but especially in the latter. Although the curves are not analogous throughout, the lymphocyte curve for radiation through ultra-violet glass (Fig. 8) is somewhat similar to that for radiation through plain glass (Fig. 4), especially as regards the large drop on the thirteenth day. This indicates that

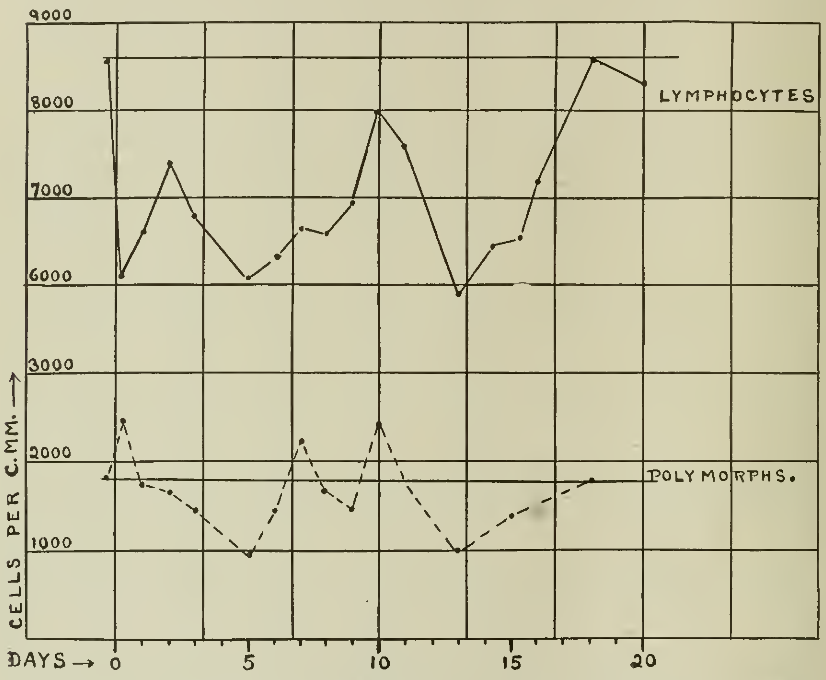

Fig. 8. Lymphocyte and polymorphonuclear counts, after exposure to iron arc through ultra-violet glass (average of three rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure ———— lymphocytes — — — — — — polymorphonuclear leucocytes.

the depressing action of the radiation through glass is largely due to the near ultra-violet in this radiation, which is not surprising as the iron arc is very rich in lines in this region.

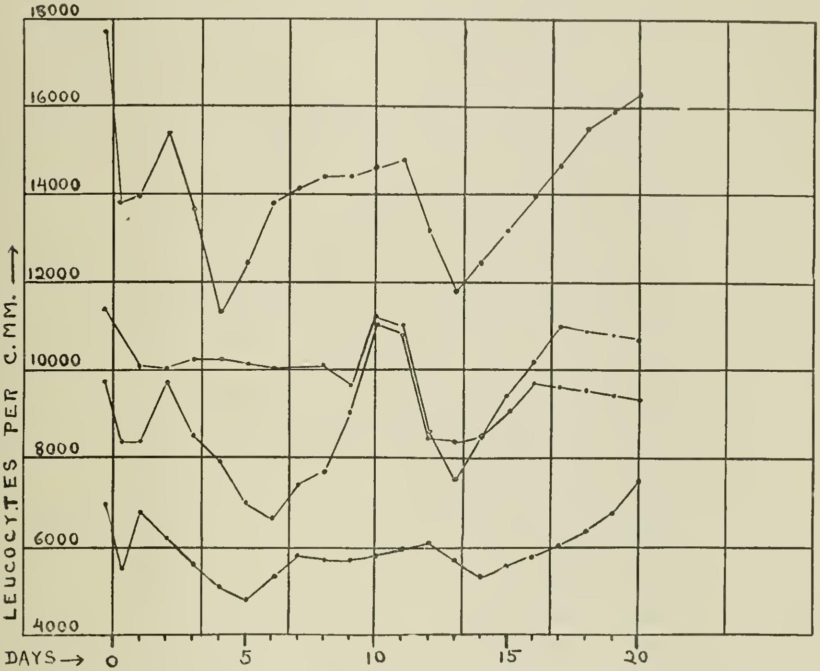

Fig. 9. Total leucocyte counts on four individual rabbits, after exposure to iron arc through ultra-violet glass. Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

Fig. 9, in which the total white counts of four individual rabbits are plotted, shows how closely they agree with each other and with the average curve.

Result of radiation with the iron arc through a picric acid screen (transmission 450 → 750 μμ) (Fig. 10).

The region transmitted by this screen includes the two yellow absorption bands of hemoglobin.

There is a moderate drop in the total count immediately after radiation, due to the combination of a large drop in lymphocytes and a small rise in polymorphonuclears. After this immediate effect the total count, the lymphocyte and the polymorphonuclear counts follow parallel curves, showing a large rise on the first and second days after

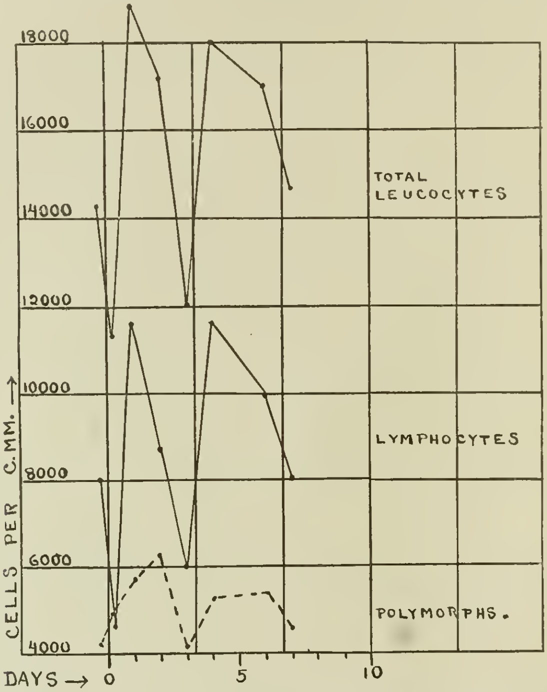

FIG. 10. Total leucocyte and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through picric acid screen (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure ———— leucocytes and lymphocytes — — — — — — polymorphonuclear leucocytes.

radiation, followed by a sharp drop on the third, an equally sharp rise on the fourth, and a return to normal on the seventh, after which no noticeable variation was found.

RESULT OF RADIATION WITH IRON ARC THROUGH RED GLASS (FIG. 11).

This resulted in an immediate fall in lymphocytes which returned to normal by the second day, and no further change was then observed. The polymorphonuclears were practically unchanged. One may conclude, therefore, that radiation of wave length longer than 650 $\mu\mu$ produces no effect other than the usual lymphocyte drop immediately after radiation.

Since there is always a drop in lymphocytes immediately after radiation, irrespective of the wave-lengths used, it seemed possible that this drop might be due to some other agency than light. A series of control experiments was performed on the animal to see whether the mere position in the box might be responsible for this drop. They were placed in the box for an hour, and blood counts were made before and after, which showed no significant change in either total or differential counts.

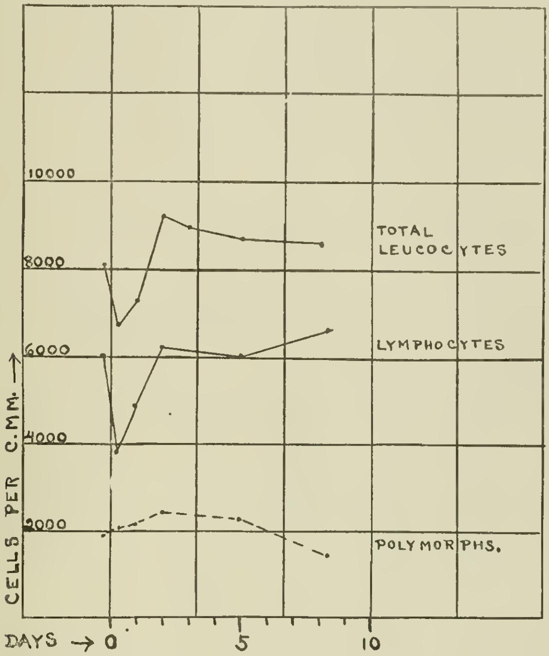

Fig. 11. Total leucocyte and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through red glass (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure. ———————— leucocytes and lymphocytes — — — — — — — polymorphonuclear leucocytes.

Effect of repeated radiation.

Radiation of one hour daily, for a week, seemed to give no greater response to the bare iron arc than a single radiation. This, however, has not been very extensively tried.

Discussion.

There is always a fall in the number of circulating lymphocytes immediately after exposure to light of any wave-length. When this is accompanied by an increase in polymorphonuclears, as in the radiation through plain glass, the total count is unchanged, but usually it results in a fall in the total count as well. Light of wave-length greater than 650 $\mu\mu$ has no effect except this usual fall in lymphocytes immediately after exposure.

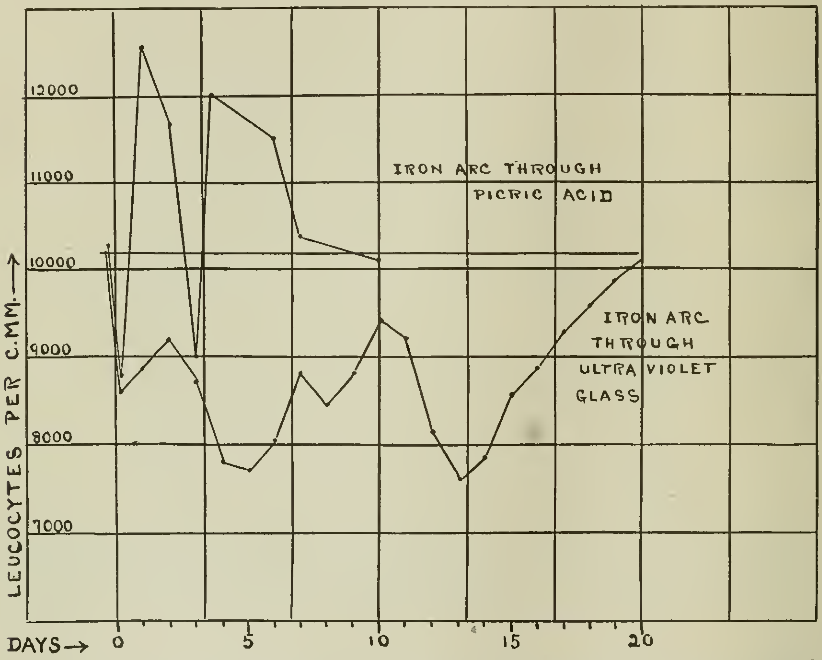

Fig. 12. Average total leucocyte counts, after exposure to iron arc through picric acid and through ultra-violet glass, showing complementary effects. Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

Light of wave-length shorter than 320 $\mu\mu$, or perhaps 300 $\mu\mu$, results solely in a great increase in lymphocytes. This confirms the suggestion, made in the previous work referred to, that the ultra-violet stimulates a lymphocytosis, leaving the polymorphnuclears constant in absolute number. It is, however, only the far ultra-violet ($\lambda < 300 \mu\mu$) which is capable of this action, as the region of ultra-violet just below the visible has a depressing effect on the lymphocytes (See Fig. 8). This near ultra-violet gives a rise in polymorphonuclears immediately after exposure, which is also found as a result of radiation through plain glass, but this is followed by a decrease below normal.

The region between 450 and 650 $\mu\mu$, however, increases both the polymorphonuclear and lymphocyte counts. From this fact a sufficient explanation can be found perhaps for the experimental observation that animals kept continually in sunlight do not as a rule show a different leucocyte count from those kept in darkness. The stimulating effect of the yellow light, and the small amount of far ultra-violet present in the solar spectrum, is offset by the depressing action of the blue, violet, and near ultra-violet as indicated in Fig. 12. In the solar spectrum the intensity of yellow light is greater than the intensity of the blue and violet, and the relative intensity is possibly such that this balancing action is practically complete, except when an unusual intensity of far ultra-violet (290 to 300 $\mu\mu$) is present, as may be the case in the tropics. In the spectrum of the iron arc through glass which is composed of somewhat the same range of wave-lengths as the sunlight, although it does not reach as far as 290 $\mu\mu$ in the ultra-violet, the intensity of the violet and near ultra-violet is relatively very much greater, and the total effect is more parallel to that of the near ultra-violet alone (See Fig. 6).

CAUSE OF EFFECT.

The cause of the action of light on blood is not known. Experiments are now in progress on this point, but as yet no satisfactory conclusion has been reached.

The invariable drop noticed in the lymphocytes immediately after radiation, furnished the suggestion that some product of the disintegration of these cells may act as a stimulus to the lymph nodes and thus account for the subsequent rise. This suggestion is not borne out by the results of radiation with the longer wave lengths, 650 $\mu\mu \rightarrow$, but it was tested directly by the following experiments, with a negative result.

An emulsion of lymphocytes in salt solution was made from the lymph glands and thymus gland of one rabbit. Half of this was injected into a second rabbit, and the rest, after being radiated in a quartz tube, with the bare iron arc, for one hour, was injected into a third rabbit. These two rabbits had had their blood counted before injection, and were then examined daily for a week. They both showed a great and immediate increase in polymorphonuclears, due possibly to the introduction of foreign protein. This was accompanied by a decrease in lymphocytes. By the next day the blood was practically normal, and no further significant change was found.

A more probable explanation is that the absorption of light by the hemoglobin or plasma of the blood may lead directly or indirectly to the formation of products that have a stimulating effect on lymphocyte production. In line with this suggestion the following experiments were made.

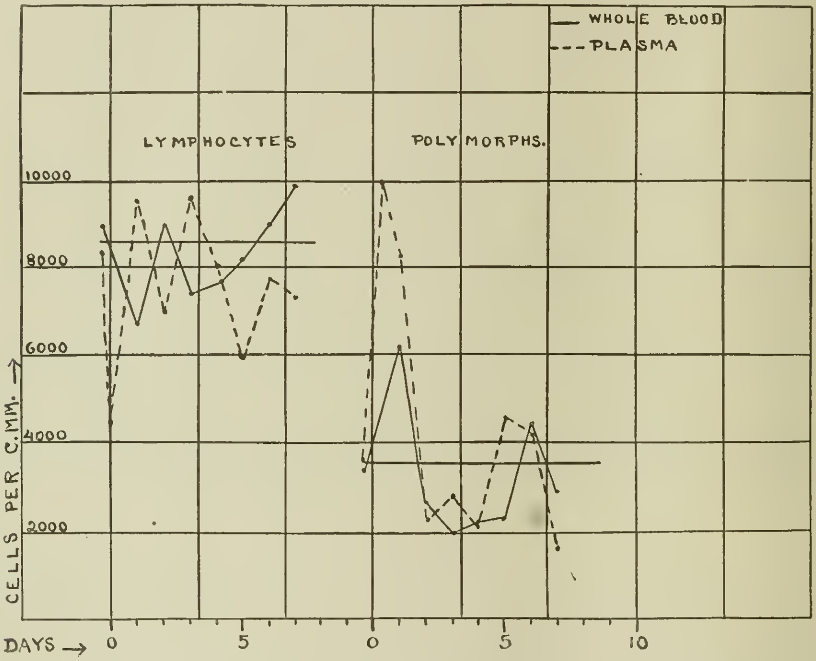

FIG. 13. Lymphocyte and polymorphonuclear counts for rabbits injected with radiated whole blood and radiated plasma. ———— injected with radiated whole blood – – – – – – – injected with radiated plasma. Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after injection.

Experiment I. 3 c.c. of blood from the heart of a rabbit were mixed with 1.5 c.c. of heparin (20 mgm. in salt solution) to prevent coagulation (22), radiated in a quartz tube with the bare iron arc for one hour, and then injected back into the same animal through an ear vein.

Experiment II. 4 c.c. of blood from the heart of a rabbit were mixed with 2 c.c. of heparin (25 mgm. in salt solution), centrifugalized, and 3.5 c.c. of clear plasma separated. This plasma was radiated for one hour in a quartz tube with the bare iron arc, and was then injected into the same rabbit through an ear vein.

The results are shown in Fig. 13. The total count in the rabbit with injected plasma showed a suggestive rise in the total count on the first day after injection. The differential, however, proved this to be due entirely to a very great rise in polymorphonuclears. Apparently the radiated plasma acted like a foreign protein. Except for this increase in polymorphonuclears the injected radiated plasma had no other stimulating effect.

The injection of radiated whole blood also gave a polymorphonuclear rise, but it was less marked than in the case of the plasma. Except for this change the action was depressing, both on lymphocytes and polymorphonuclears. The stimulating effect of the far ultra-violet on lymphocyte formation is apparently not due to an action resulting directly from the absorption of light by the hemoglobin or plasma, although no general conclusion can be drawn from this single experiment.

As it is known that ultra-violet light exerts a direct effect on the skin, leading to sunburn or marked inflammatory changes, it is possible that its action on this tissue may be responsible indirectly for the lymphocytosis. As bearing upon this suggestion an attempt was made to determine the effects of radiating a bloodless ear. In three rabbits the common carotid artery on one side was exposed under local action of cocaine and clamped. The corresponding ear was then exposed for an hour to the bare iron arc. The experiments were not satisfactory. It was found as a matter of fact that clamping the carotid did not suspend entirely the circulation in the ear and, in addition, while aseptic precautions were attempted in the slight operation involved, it was not found possible to avoid opportunities for infection. Further experiments along this line will be reported in the future.

SIGNIFICANCE OF THE ACTION OF LIGHT ON BLOOD.

In his work on animals with lymphocytosis, induced by exposure to dry heat, Murphy (23) has shown that mice with a lymphocytosis show a high degree of immunity to certain transplantable cancers, and also a very great increase in their resistance to doses of bovine tubercle bacilli. The resistance to tuberculosis is increased from two to three fold. Amoss, Taylor, and Witherbee (24) also showed that monkeys with greatly reduced lymphocyte counts, as a result of exposure to x-rays, show a much increased susceptibility to an intracerebral inoculation of poliomyelitic virus filtrate. From these results it would seem that any method of inducing a lymphocytosis (heat or far ultra-violet light) would aid in maintaining a high resistance to these diseases at least. It has been suggested, in fact, by Rollier (25) and others that the beneficial effects of heliotherapy in tuberculosis may be due to the action of the actinic rays in increasing the lymphocyte count. Chamberlain, Wickline and others (11, 12, 13, 14, 15), working on the blood count of soldiers in the Philippines, and finding, as a result of the tropical climate, an increased lymphocyte and a decreased polymorphonuclear differential count, took this to indicate a poor physical condition on the part of the men. The chief phagocyte cell of the blood is the polymorphonuclear neutrophile and it is presumably of great importance in the destruction of bacteria in the blood stream. Wickline (12) quotes Cabot as saying ''It would appear that the degree of health in persons not organically diseased might perhaps prove to vary directly with the percentage of polymorphonuclear cells in the blood.'' In Wickline's work (12) the total leucocyte count remained practically constant throughout the nineteen months of examination while the percentage of polymorphonuclears steadily fell, and the percentage of lymphocytes steadily rose. There was therefore an absolute decrease in polymorphonuclears, and this condition may signify an increased susceptibility to bacterial invasions.

If a condition may be obtained in which the absolute number of polymorphonuclears remains practically normal while the absolute number of lymphocytes is greatly increased, as after exposure of rabbits to a bare iron arc, the blood would have normal phagocytic power together with such increased resistance as may depend upon the peculiar activity of the lymphocytes.

Conclusions.

Direct radiation of rabbits' ears for short periods (one hour) by an iron arc through different filters has shown:

1. The region of far ultra-violet (wave length shorter than 300 $\mu\mu$) has practically no effect on the absolute number of polymorphonuclears, but produces a very marked lymphocytosis, lasting about three weeks.

2. The near ultra-violet (330 to 390 $\mu\mu$) has a marked depressing effect on the lymphocytes, and, to a less degree, on the polymorphonuclears.

3. The region between 450 and 650 $\mu\mu$ has a stimulating action on both lymphocytes and polymorphonuclears, particularly on the former.

4. The wave-lengths longer than 650 $\mu\mu$ (the red and infra-red), produce no effect on the blood beyond the drop in lymphocytes which occurs immediately after exposure to light of any kind.

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AN EXPERIMENTAL STUDY OF THE INTRACRANIAL PARASITISM OF THE HUMAN LUNG FLUKE, *PARAGONIMUS WESTERMANII*.*

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INTRODUCTION.

Cerebral parasitism by the human lung fluke, *Paragonimus westermanii* was first recognized by Otani (1888–1889) in 1887. This author described the case of a man who, after suffering for a long time with paragonimiasis of the lungs, became suddenly afflicted with general epilepsy. The patient died after a lapse of one and one-half years, and the autopsy disclosed one tumor, as large as a hen's egg, and several smaller ones in the occipital and the frontal lobes of the brain. In the larger tumor were found two adult lung flukes and some yellowish brown material, consisting of many eggs of the worms, Charcot-Leyden crystals and hematoidin crystals. Afterwards (1890–1891) he reported the clinical histories of three similar cases.

Following this work 19 additional cases have been reported by Otani (1888), Katsurada (1900), Taniguchi (1904), Misumi (1907), Musgrave (1907), Tsunoda (1908), Karasawa (1910), Kimura (1913), Inamoto (1917) and Kawamura (1918). In all of these the presence in the brain, of the parasite or its eggs was established at autopsy. The authors mentioned have discussed particularly the relation of this type of parasitism to Jacksonian epilepsy and other prominent nervous symptoms. An analysis of these cases will be given later in this paper.

The hypothesis that the worms and their eggs were carried from the lungs to the brain by means of the blood, was formerly accepted by all authors. The pathological changes in the brain were believed to be due to the production of anemic infarcts referable to emboli of eggs or worms (Katsurada (1900), Taniguchi (1904), Tsunoda (1908), Yamagiwa (1892). The senior author (Yokogawa, 1916) in an extensive series of experiments has proved, however, that the lung fluke in its migrations in the final host always travels through the soft tissues and is never carried by the blood stream. In the course of these experiments he found four partially developed worms traveling through the loose connective tissue along the blood vessels and nerve trunks in the necks of two dogs. The position of these worms suggested that the course of the parasites from the chest cavity to the cranial cavity is along the soft connective tissue of the neck and through the jugular foramen. Later Kawamura and Matsui * (1916) and Ando (1917) also reported the finding of worms, in their own experiments, in the same location as those which Yokogawa found. No one, however, had been successful in producing intracranial parasitism by this worm experimentally. The experiments described in this paper were undertaken for the purpose of studying the pathological changes produced in the brains of experimental animals as a result of cerebral paragonimiasis, and also with the hope of discovering the course which the lung fluke takes in migrating to the brain.

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* A contribution from the Department of Pathology of the Medical College of Formosa and the Department of Medical Zoology of the School of Hygiene and Public Health of The Johns Hopkins University.

All the dogs, used in these experiments came from the city of Taihoku, Formosa, where the work was carried on, or from the districts close to this city. Previous examinations had shown that *Paragonimus westermanii* is rare among the dogs of this region. As an additional precaution, however, the dogs were kept for a sufficient length of time to allow any parasites that might have been present to develop to maturity. A series of fecal examinations for the eggs of the parasite was made in the case of each dog before it was used for the experiment. Further, since the degree of development of the parasites for various periods of time had been established by previous experiments it was possible to check the source of any parasites found in the course of the experiments by the degree of growth at the time of observation. It would accordingly seem extremely improbable that the results obtained could have been complicated by previous infection of any of the dogs.

Three series of experiments were carried out: The first series was performed with the idea of ascertaining whether the course of the fluke toward the brain was by way of the connective tissues, and whether entrance into the cranial cavity was effected through the foramina. The second series of experiments was made to test the hypothesis held by certain authors that in cases of cerebral parasitism by the lung fluke the parasite is carried to the brain by the blood stream. In the third series of experiments both immature and adult flukes were introduced directly into the cranial cavity by trephining the skull, for the purpose of determining what pathological changes they would produce in the brain and what course they would follow in their migrations. An analysis of the case records of human cerebral paragonimiasis has finally been made for the purpose of ascertaining whether the data regarding the pathological changes produced in the brain and the position of the parasites furnish any clue to the course which they follow in migrating to the cranial cavity.

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* This was reported by Dr. Kawamura at the meeting of the Japanese Pathological Society in Tokyo, in April of 1916.

I. EXPERIMENTS TO TEST WHETHER IMMATURE LUNG FLUKES WILL ENTER THE CRANIAL CAVIITY THROUGH THE ANATOMICAL APERTURES.

Experiment 1.—Immature distomes which had been undergoing development for varying periods of time in other animals were introduced into wounds in the sides of the necks of five dogs. The number of flukes used for each experimental dog and the age which they had reached in other animals was as follows: for dog number one, eleven flukes, twenty days old; for dog number two, eleven flukes, twenty days old; for dog number three, eighteen flukes, eighteen days old; for dog number four, nine flukes, twenty-three days old; and for dog number five, fifteen flukes, forty-two days old. The dogs were examined at intervals varying from one to fifteen days following the operation, but none of the young distomes had entered the cranial cavity. In three of the dogs, however, worms were found in the pleural cavity, at the time of examination. The negative results of this experiment while suggestive do not prove that the lung flukes may not enter the cranium through the anatomical apertures, since the experimental conditions were, of course, very different from a natural process, and since the interval between the introduction of the flukes into the dogs and the examination was a short one owing to the development of severe suppuration in the area of operation.

Experiment 2.—Young distomes which had just emerged from their cysts were introduced into the orbits of eight dogs, through the conjunctiva, to determine whether they would enter the cranial cavity by passing through the superior orbital fissure or the foramen nervi optici. Table I gives the data obtained in this experiment. Although this experiment was not far removed from a natural process, in no case were the worms found to have penetrated into the cranial cavity. The failure of the young distomes to take this course is probably due to the fact that the orbital walls in dogs are incomplete. This gives the worms a chance to escape through the soft tissues of the external wall of the orbit, without penetrating the cranial cavity. That this is actually what happened is indicated by the finding of worms in the pleural cavities and lungs of five of the eight dogs (see Table I).

TABLE I.

Data obtained as the result of the introduction of worms which had just escaped from their cysts, into the orbits of dogs.

Serial number of dogs used.	Number of young worms introduced into orbits.		Number of days from introduction of worms until examination of hosts.	Locations in which worms were found at examination.
	Right orbit.	Left orbit.		
I.......	20	8	5 days	In left orbit
II.......	10	10	11 days	In left orbit
III.......	—	20	15 days	One in pleural cavity
IV.......	13	17	22 days	None found
V.......	28	21	68 days	Two in pleural cavity
VI.......	50	64	74 days	Two in pleural cavity
VII.......	22	23	127 days	Six in lungs
VIII.......	44	39	201 days in right orbit, 177 days in left orbit	Three in pleural cavity

II. EXPERIMENTS UNDERTAKEN TO DETERMINE WHETHER YOUNG LUNG FLUKES, JUST FREE FROM THEIR CYSTS, CAN BE INTRODUCED INTO THE CRANIAL CAVITY BY WAY OF THE INTERNAL CAROTID ARTERY.

Young distomes, which had just escaped from their cysts were placed in normal saline and injected into the common carotid artery, of eight dogs. The results obtained are shown in Table II. In order to insure that these worms would enter the internal carotid artery and thus be carried to the brain a ligature was placed upon the common carotid artery just beyond the lingual branch in dogs IV and V, while in dogs VI, VII and VIII all the branches of the common carotid artery except the internal carotid artery were tied.

As shown in Table II no worms were found in any case in the cranial cavity, while in four of the eight dogs they were met with elsewhere in the body. The findings in the last 3 cases, as recorded in this table, are especially significant, as they leave no doubt that the large number of worms injected was actually carried by the internal carotid artery to the brain. There were no gross pathological changes produced in the brains of any of the dogs used in this series of experiments. That the worms had actually entered the brain substance was shown by a thorough microscopical examination of the brain of the last dog, which disclosed hemorrhagic infiltrations and many irregular areas of destruction of the cerebral substance. These

TABLE II.

Data from the experiments on the introduction of young flukes, just freed from their cysts, into the cranial cavity through the internal carotid artery.

Serial number of dogs used.	Interval between the introduction of the worms and the examination of the dogs.	Number of worms injected.	Place of injection.	Arteries ligated.	Location of worms found.
I....	8 days	13	Left common carotid artery	—	—
II....	270 days	32	Left common carotid artery	—	8 worms in the pleural cavity
III....	24 days	18	Left common carotid artery	—	—
IV....	27 days	43	Right common carotid artery	Common carotid artery beyond lingual artery	6 in the pleural cavity, 1 in the abdominal cavity
V....	77 days	45	Left common carotid artery	Common carotid artery beyond lingual artery	—
VI....	15 days	117	Left common carotid artery	All branches of the common carotid artery, except the internal carotid artery	—
VII....	12 days	112	Right common carotid artery	All branches of the common carotid artery, except the internal carotid artery	1 in the pleural cavity
VIII....	46 days	89	Left common carotid artery	All branches of the common carotid artery, except the internal carotid artery	3 in the pleural cavity

changes could only have been produced by the young distomes in the brain. These findings therefore raised three questions: (1) Why were there no gross pathological changes in the brains; (2) why did the worms not remain and develop in the cranial cavity, and finally (3) by what route did they travel from the cranial cavity to the chest cavity? An attempt will be made in the remainder of the paper to answer these questions.

III. EXPERIMENTS MADE TO DETERMINE WHAT CHANGES TAKE PLACE IN THE BRAIN, WHEN SPECIMENS OF *Paragonimus westermanii*, IN VARIOUS STAGES OF DEVELOPMENT, ARE INTRODUCED DIRECTLY INTO THE CRANIAL CAVITY OF DOGS BY TREPHINING THE SKULL, AND WHAT COURSE THEY TAKE IN MIGRATING FROM THE CRANIAL CAVITY TO THE CHEST CAVITY.

Since it has been found impossible to produce intra-cranial infestation experimentally either by the introduction of the young worms into wounds in the necks of dogs, or by way of the orbits, or by the internal cartoid artery, a series of experiments was undertaken, in which both young and fully developed worms were introduced directly into the cranial cavity by trephining the skull. The technic followed was that usually employed in such operations.

After exposure of the dura mater, this membrane was incised and the worms were placed upon the pia mater. The cut edges of the dura were held apart and the worms observed until they had disappeared through the pia mater. The opening in the skull was then closed with paraffin and the skin sutured. In introducing the adult worms the operation was the same, except that the dura mater had to be raised a little to make a space for the worm between it and the pia mater. In all, six dogs were used in this experiment. On account of the special significance of the findings obtained in these experiments a detailed account will be given of each.

Experiment 1.—On December 17, 1918, 45 lung fluke larvae, just freed from their cysts, were introduced under the dura mater of a dog by following the operative procedure described above. On January 9, 1919, i.e., after about 23 days, the dog was found dead. An examination showed that the wound made at the operation was completely healed, and that the opening in the skull was also entirely closed by the paraffin. A part of the dura mater, at the point of operation appeared roughened, a little thickened and congested. No striking changes were noted in the brain substance, although there was a little congestion. We could not find any worms in the cranial cavity. Two distomes, however, were encountered in the pleural cavity. On the surface of both lungs there were several ecchymotic points, and in the lungs several small greyish resistant areas without air.

Experiment 2.—A dog was operated on December 17, 1918, and 36 larvae, just free from their cysts, were introduced under the dura and the wound closed, as described above, after the worms had been seen to penetrate into the brain, through the pia mater. On January 15, 1919, i.e., 27 days after the operation, the dog was killed, and carefully dissected. A part of the dura mater which corresponded to the trephined area was found firmly adhering to the skull, and contained a number of grey or yellow granules. There were no striking changes in the brain except several small traumatic areas on its surface, which corresponded to the place of the operation. In the water, in which we washed the brain, was found a single distome, and eighteen more distomes were encountered in the pleural cavity. The worms found in the pleural cavity were in the normal stage of development corresponding to the time represented in this experiment, but the single specimen in the brain, although introduced at the same time as the others, was very much smaller. Both lungs were somewhat congested and showed many irregular areas of ecchymosis on their surface.

Experiment 3.—A dog was operated on December 20, 1918, and 16 distomes, which had just emerged from their cysts, were introduced under the dura mater, as in the preceding experiments. On January 15, 1919, i.e., 26 days after the operation, this dog was killed. We could not find any worms either in the cranium or in the chest, nor could we notice any changes in the brain caused by the distomes.

Experiment 4.—On February 27, 1919, a dog was operated on and 18 larvae which had just emerged from their cysts were placed under the dura mater, as in the previous experiments. This dog was killed on March 4, only 5 days after the operation. The wound was not yet completely healed. The membranes and the brain itself showed no striking changes, although there was a somewhat greater degree of congestion than normal. No distomes were found in the cranial cavity, but one was met with in the pleural cavity.

Experiment 5.—A dog was operated on March 4, 1919, three full-grown distomes which had developed in a cat, were introduced under the dura mater, and the wound closed. The dog was killed on the seventeenth of the same month, i.e., 13 days after the operation. Although the wound had suppurated and a purulent inflammation, the result of the operation, had developed in the right temporal muscle, yet the opening made by trephining was found to be closed by a membrane of connective tissues. On opening the cranium, we found that the skull contained much blood. The dura was generally congested and adhered firmly to the skull in the right parietal region, and to the pia mater of the same region. This adhesion corresponded to the area which had been trephined, and appeared a little thickened and opaque. There was an ecchymotic area, the size of a one cent piece, in the cortical substance of the right frontal lobe, and another as large as a pea, in the cerebral substance in the middle region of the right parietal lobe. Other parts of the cerebrum, as well as the cerebellum and the medulla oblongata, showed no striking changes. Both lungs were free from adhesions, but in the posterior upper border of the lower lobe of the left lung, we felt a resistant area which seemed embedded in normal tissue. The surface of the lung in this region was smooth and normal. When the resistant area was dissected out we found a circumscribed hemorrhagic cyst, containing a full-grown distome. There was no development of connective tissues in the wall of this cyst.

Experiment 6.—A dog was operated on March 4, 1919, and three full-grown distomes which had developed in a cat, were introduced under the dura mater. This dog was killed on the seventeenth of the same month, i.e., thirteen days after the operation. The wound had healed completely. There was a small area of ecchymosis in the right temporal muscle. The bone and the dura mater were hyperemic. The sagittal sinus, especially on its right side, was highly congested. The right half of the dura mater adhered slightly to the skull by fibrous tissue and in the parietal region of the same side was found a dark brown spot as large as the tip of a man's finger. The pia mater was also congested, especially over the anterior half of the right parietal lobe. The external surface of the temporal lobe adhered widely to the dura mater, and contained a greyish lump. Although this lump gave the appearance of a dead distome, we could not be certain of this from examination. The substance of the brain was a little softened, but there were no other striking changes. On the inferior surface of the upper lobe of the left lung there was an ecchymotic area, as large as a pea, and on the upper part of the posterior surface of the left lower lobe, one as large as the tip of a man's thumb. In the latter region we felt a resistant area which contained a full-grown distome. Another full-grown worm was found in the right pleural cavity and in the right lung there were several ecchymotic spots of various sizes, which were apparently caused by this worm.

In Table III is presented a summary of the six experiments described above, including a statement of the place on the skull where the operation was performed.

TABLE III.

No. of experiment.	Duration of infestation.	Number of larvae introduced.	Adult worms introduced.	Trephined area.	Gross pathological changes in the brain.	Location of worms found.
I......	23 days	45	—	Left parietal region	—	2 in the pleural cavity
II......	29 days	36	—	Left parietal region	—	18 in the pleural cavity; 1 in the brain
III......	26 days	16	—	Left frontal region	—	—
IV......	5 days	18	—	Left frontal region	—	1 in the pleural cavity
V......	13 days	—	3	Right parietal region	+	1 in the lung
VI......	13 days	—	3	Right parietal region	+	1 in the lung; 1 in the pleural cavity

An analysis of the experiments just described makes it possible to draw certain definite conclusions regarding intra-cranial infestation on the part of *Paragonimus westermanii.* One of the most surprising findings is the fact that the pathological changes which result from the introduction of the worms into the cranial cavity of dogs, are remarkably slight, especially when compared with the changes that have been described by various authors in cases of cerebral paragonimiasis occurring in man. Two explanations might be offered to account for this difference. In the first place, the time spent by the worms in the brains of the dogs was very short, while in the human cases there is every indication that the condition was of much longer standing. In the second place the tissues of the dog, especially the brain, do not react as strongly to injury as do those of man. This is shown by the fact that the trephining operation is so much less severe for the dog than for man. On the day following such an operation the animal will stand up and begin to take food.

Another point which is clearly brought out by these experiments is the fact that the cranial cavity is not a favorable location for the development of *Paragonimus westermanii.* This view is of course strongly supported by the relative infrequency of cerebral paragonimiasis in man. The finding, in the second experiment of the series, of a young distome in the cranial cavity, twenty-nine days after the operation throws light on this question. This worm showed considerable retardation in its development, being much smaller than those of the same age which were found in the pleural cavity of the same experimental dog. The rapidity with which the worms introduced into the cranial cavity migrated from this location also suggests that it is an unfavorable environment for their development.

As in the above experiments the worms which were introduced into the cranial cavity were later found in the pleural cavity or the lungs, the question of the course which they followed in this migration is of course immediately raised. There are two possible routes which suggest themselves: (1) The worms may have left the cranial cavity by way of such anatomical apertures as the posterior fissure or the inferior orbital fissure and may have reached the chest cavity by traveling through the soft tissues, especially the connective tissue along the large blood vessels or nerves; or, (2) they may have gotten into the cerebral sinuses and have been carried to the lungs by way of the internal jugular veins, the right ventricle and the pulmonary arteries. It was found that the young distomes which were introduced into the orbits of dogs and which had migrated to the pleural cavity and the lungs, without entering the cranium, needed from 8 to 15 days to complete their journey. This being the case, a longer period of time even would seem to be necessary for worms, placed on the surface of the brain, to reach the lungs, if they escaped from the anatomical apertures and migrated through the tissues of the head and neck. In this connection experiment No. 4 is of special interest. In this case only five days elapsed from the introduction of the worms into the cranial cavity until one was recovered from the pleural cavity. In the light of previous knowledge it would seem to be impossible that migration through the tissues could be accomplished in such a short time.

Of further significance in this connection are the findings recorded in the case of the fifth experimental dog. In this animal a full grown distome was found in a fresh hemorrhagic cyst, situated near the upper posterior edge of the lower lobe of the left lung. The condi-

TABLE IV.

Analysis of the position in the brain of the pathological changes found in the reported cases of human cerebral paragonimiasis.

Reporter			Otani, S.	Katsurada, F.	Taniguchi, N.	Misumi, J.			Tsunoda, G.	Karasawa, M.	Inamoto, K.								Kimura, D.	Kawamura, R.	Total 18.	
Serial number of cases......			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII		
Location of foci of infestation with *Paragonimus westermanii* in the human brain.	Frontal lobe	Left				+					+									+	3	9
		Right	+		+				+		+					+				+	6	
	Parietal lobe	Left					+				+			+							3	5
		Right			+				+												2	
	Temporal lobe	Left		+		+	+		+			+				+		+		+	8	12
		Right			+				+	+	+										4	
	Insula	Left																				2
		Right						+		+											2	
	Occipital lobe	Left				+	+					+	+		+		+	+	+		8	15
		Right	+		+			+		+		+	+					+			7	
	Thalamus and nucleus	Left																				2
		Right							+		+										2	
	Cerebellum	Left																+			1	1
		Right																				
Existence of worms.......			+				+		+												3	

tion of the cyst, especially the absence of a connective tissue wall, indicated that the worm had but recently reached this location. The surface of the lung was entirely normal. It is evidently impossible that an organism of this size should have penetrated through the surface of the lung from the pleural cavity without producing an obvious break. Therefore the only way in which this case can be explained is by assuming that the worm entered the lung from within, by means of the blood stream. A study of the cerebral sinuses in the dog increased the probability of this course. These sinuses are quite large and situated between the two layers of the dura mater in places of less intimate contact between the cranium and the brain. The cerebral surfaces of the sinuses are made up of a network of loose connective tissue, which would make the entrance of the lung flukes very easy. Once in the sinuses they would be very readily carried along the large internal jugular veins to the heart. This view of the migratory course makes it possible to explain why amongst all the cases in which the lung flukes were introduced into the cranial cavity, both by way of the internal carotid artery and by trephining, only one specimen was recovered inside the cranium, while all the other specimens found were obtained either from the pleural cavity or the lungs. Whenever the worms were found in the pleural cavity the surfaces of the lungs showed the effects of their passage.

IV. An analysis of the cases of cerebral paragonimiasis reported in man.

In the experiments outlined above we have failed to show how the pathological changes are produced in cerebral paragonimiasis, and what course the parasites take in their migration into the cranial cavity. In Table IV an analysis is presented of the position in the brain of the pathological changes that have been found in the reported cases of human cerebral paragonimiasis, in the hope that some light may thus be shed on these two unsolved questions.

The main pathological changes found in all these cases except that of Musgrave (1907)* are quite similar. They consist of large cysts undergoing necrotic softening in the medullary substance, and some areas of necrobiosis, or of sclerotic foci containing many eggs. These pathological changes are so marked that it seems certain that they must have been produced as the result of invasion on the part of the worms, and by the eggs excreted by the worms themselves during these invasions, and not merely by eggs carried to the brain from elsewhere, as believed by some authors. Adult worms, however, were present only in three of these cases. The extensive pathological changes in those cases where no worms were present can be explained only, if we accept the view that the flukes invaded the brain and then made their way out again. This view is supported very strongly by the evidence brought forward in Section III of this paper which shows a method by which adult worms which have entered the cranial cavity may escape by way of the cerebral sinuses. In some cases where the worms were not found it is possible that they may have died in the unfavorable surroundings of the cranium, and become unrecognizable as the result of post mortem changes. At any rate, it seems impossible that such changes as those which have been described in the human cases of cerebral paragonimiasis, as for example, the foci or pathways containing eggs could have been produced in any other way than by the presence of adult worms in the cranial cavity.

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* Musgrave's description of the pathological findings in his case is as follows: "The membranes of the brain are thickened and adherent in many places; bound in these adhesive masses are cystic, parasitic abscesses similar to those of the pleural cavity. The choroid plexus is coarse and granular, slightly blueish and a few eggs were found in its substance. The brain substance itself appeared to be normal, with the exception of a moderate congestion near the cortical surface. No distinct abscess cavity was found in this organ."

Further, from the table it will be seen that pathological foci in the brain were always situated in the temporal or the occipital lobes, except in Inamoto's case (1915) in which there was only a small parasitic abscess in the left parietal lobe. In every case of cerebral paragonimiasis also some of the pathological foci in the brain were connected with the meninges either directly or through an interposed infiltrated area. From this position of the pathological areas in the brain we can infer that the worms penetrated into the cranial cavity through the foramina situated at the base of the skull. Although there are many apertures at the base of the skull, the jugular foramen seems to be most convenient for the entrance of the parasite, because it is very large and is not completely filled by blood vessels and nerve trunks and through this opening the worms could easily have reached the positions where the pathological changes have been noted in the case records. The finding, by the senior author, in two experimental dogs, also, of partly grown flukes in the neck region, apparently traveling forward in the connective tissue surrounding the blood vessels and nerves which pass through the jugular foramen, is suggestive that this opening is the essential passageway into the cranium.

We may conclude therefore, that the pathological changes in the brain are almost always caused by the entrance of partly grown or adult worms through the foramina at the base of the skull, and their subsequent invasion of the brain tissue, and not by emboli composed of eggs. The exceptional case of Musgrave, however, can only be explained by the supposition that the pathological changes were produced by emboli of eggs, since he found some eggs in the choroid plexus without striking changes in the brain.

Thanks are due to Dr. W. W. Cort of the Department of Medical Zoology of the Johns Hopkins University for his help in revising the manuscript. We also wish to express our thanks to Dr. S. Murai, Assistant Professor of Surgery in the Medical College of Formosa for his aid in performing the operations on the experimental dogs.

SUMMARY.

1. Immature specimens of *P. westermanii* which had been developing for varying periods of time in other animals did not migrate into the cranial cavity of dogs when introduced into wounds in the sides of their necks.

2. Young distomes newly emerged from their cysts did not penetrate into the cranial cavity when introduced into the orbits of dogs, but in same cases made their way into the pleural cavity.

3. Young distomes which had just escaped from their cysts when injected into the common carotid of dogs under conditions which precluded the possibility of their not being carried into the cranial cavity were never found in the cranium, but in several cases were encountered in the pleural cavity.

4. When specimens of *P. westermanii,* both larvae and adults were introduced directly into the cranial cavity of dogs, by trephining the skull, only one out of of over one hundred worms was found in the cranial cavity, but a number were met with in the lungs or pleural cavity.

5. The course which these worms followed from the cranial cavity to the lungs and pleural cavity was probably by way of the cerebral sinuses, the internal jugular veins and the pulmonary arteries.

6. That the cranial cavity is an unfavorable location for the development of *P. westermanii* is suggested by the relative infrequency of cerebral paragonimiasis in man, the finding of a worm in this location showing retardation in development, and by the rapidity with which the worms introduced into the cranium migrated from this location.

7. An analysis of the human cases of cerebral paragonimiasis indicates that in the great majority the pathological changes are produced by the entrance of partly grown or adult worms through the formina at the base of the skull, and their subsequent invasion of the brain tissue, and not by emboli composed of eggs.

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ON THE RELATIONSHIP OF INFECTION BY HOOKWORM TO THE INCIDENCE OF MORBIDITY AND MORTALITY IN 22,842 MEN OF THE UNITED STATES ARMY AT CAMP BOWIE, TEXAS, FROM OCTOBER 1917, TO APRIL 1918.

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INTRODUCTION.

The following investigation of the quantitative effect of the presence of infection by hookworm upon the incidence of disease and the resulting burden of sickness, as represented by regimental reports of sick call and by hospital admissions, is based upon data collected at Camp Bowie, Texas, in April, 1918, by the Laboratory Car Metchnikoff, Major Charles A. Kofoid, Sanitary Corps, U. S. A., in charge. The data were collected in the course of a hookworm survey of the 36th Division, U. S. Army, incorporating former National Guard organizations from Texas and Oklahoma, and brought to its full complement by infiltration of recruits from the same general localities. It had almost reached its full numbers at the time of the survey. The total number of men on rosters furnished was 25,224, including some units belonging to the Camp proper, rather than to the Division. Of these, 1,565 or 6 per cent. were not surveyed. Of the 23,659 examined 2,921 or 12.3 per cent. were found to be infected with hookworm, presumably *Necator americanus* in most cases, as there were relatively few men of European birth in the Division. The greater part of these infections were light and not attended by the manifest clinical symptoms constituting hookworm disease.

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In one regiment only, the 143d Infantry, recruited mainly from Eastern and Southern Texas, did the infection exceed 20 per cent., rising in that organization to 30 per cent., and in companies C, D, and E thereof, to 68, 63, and 54 per cent. respectively.

The aim of the investigation was to determine by a statistical study the extent of sickness in the Division, as recorded in the regimental sick call and in the hospital admissions, in the two groups of men separated by our hookworm survey, namely the men with hookworm and those in which this had not been detected, and to find a quantitative expression for the increased burden of sickness which this infection was reputed to place upon the army.

The Camp records, showing the incidence of sickness, which were used in the compilation of our data, covered the period from October 1, 1917, to about April 15, 1919, and are thus partly representative in that they cover a period of time sufficient to afford an adequate volume of data. They include the winter season with its incidence of respiratory diseases, and the measles–pneumonia epidemic of the winter of 1917–1918.

Acknowledgments.

This investigation was made possible only by the cooperation of many officers and enlisted men. The multitude of the data involved, and the necessity of supervision and inspection, arising from the varied nature of the original data, have required the assistance of collaborators. The initiation of the investigation was due to the prevalent opinion at Camp Bowie that regiments from the area of heavy hookworm infection had been particularly subject to sickness, lax in discipline, and of lowered efficiency. The lively interest of Brig. General Greble in Command of Camp Bowie, and the efficient cooperation of the Division Surgeon, Colonel Metcalf, and of Colonel Hansen of the Base Hospital, greatly facilitated securing the original data in satisfactory form for use.

Especial mention should be made of the services of Captain M. A. Barber, Sanitary Corps, then of the Laboratory Car Metchnikoff, for his deep interest in and efficient supervision of the preliminary assembling and tabulation of the original data. In this work 1st Lieutenant George H. Bigelow, M. C., 1st Lieutenant E. S. Maxwell, M. C., 1st Lieutenant R. S. Robinson, M. C., 1st Lieutenant H. B. Yocom, S. C., and Sergeant T. I. Storer of the Laboratory Car Metchnikoff, gave efficient aid.

The final computations, tabulations and re-inspection of the data were made at the Army Laboratory, Port of Embarkation, by a group of technicians and enlisted men, drawn from the staff and personnel of the laboratory, under the direction of the authors. Much of the laborious assembling of the data has been done by Miss J. N. Wasserman and Miss M. Gimbel of this Laboratory.

METHODS.

The data for this investigation were compiled from two sources, the records of the Regimental Surgeon and of the Base Hospital, on cards, based on those used by Majors Siler and Cole (1917) in their hookworm survey of three regiments at Fort Sam Houston, Texas.

The form of the card is as follows:

Surname Christian	Rank	Co.	Organization	Date enlisted	No.
Residence prior to enlistment State	County	Town			
Occupation	Where spent last 10 yrs. Orient? Tropics?				
Height Age	Clinical symptoms				
Previous treatment for hookworm. When?					
Examination for hookworm: Date of sample:	No. of times on sick report: Date and diagnosis:				
Date of examination: Date of treatment:	Treated in hospital: Diagnosis:				
Date of reexamination: Result of reexamination:	Other parasites:				

These cards were filled out at the regimental infirmaries, under the supervision of the regimental surgeons, in all sections except those referring to the results of examination, clinical symptoms, and hospital record. The results of the examination were entered on the card by the examiner at the laboratory, and the hospital record was compiled from the data on file at the Base Hospital.

The entry for ''times on sick report'' was made in number of days, but the hospital record was entered only as ''admissions or times sent to the hospital.''

In a number of cases, not exceeding 5 per cent. of the records for sick call, the data returned were in indefinite terms such as ''continually,'' ''chronic,'' ''many times,'' ''repeatedly,'' ''several times,'' etc. In all such entries we have arbitrarily set the numerical value of the sick calls at 10, this number being much below the actual number of calls recorded, of men who might be characterized by the phrases that we are interpreting.

THE PROBABLE ERROR.

In all statistical work the mathematical statement of the probability of error may be computed and expressed quantitatively, on the basis of the quantitative factors entering into the problem. In the investigation here reported certain other factors not expressed quantitatively in our data, so far exceed in importance the available quantitative data, that any formal computation of the probable error would be inadequate and perhaps misleading.

Since the reports for sick call are made by different regimental surgeons, and the policy in the matter of reporting men on sick call varies with the individual surgeon and the pressure of military exigencies, the data are subject in this respect to an indeterminate variable. The nature of this variable is such that it tends in the main to reduce the sick calls, and since our evidence shows that the sick calls are greater among men infected with hookworm than in those in whom it was not detected, the net result of this variable will be to reduce the volume of the evidence for the effect of hookworm, but not to change the direction of the differences detected.

Another source of error is that resulting from the transfer of men from organization to organization. In a changing military unit, such as a division in process of organization, the volume of the movement of men from the detention camp to their final allocation is considerable. Since the record of sick calls is a company record, and does not follow the man in transfer, it results that our data fail to include the sick calls and hospital records of men prior to the date of their last transfer. The clerical burden of determining the total record of sickness of every man in the existing system of medical records would have at once prevented this investigation, had it been thus attempted.

Our records therefore show only a part of the total sickness in the 36th Division. The part included in the different organizations varies with the number of transfers, their date, and the age of the organization. It is well known that the more efficient men of an organization are less liable to transfer. The net result of this tendency is that men with hookworm who are, as a rule, less efficient, are more liable to transfer, and in consequence their accumulated sick call is left behind, and their previous hospital record could not be recorded against them in our inspection of the hospital files, because of the absence of any clue readily available, as to their present affiliation. As in the case of the variability of standards of sick call, so also here, this incompleteness in our data tends to reduce the volume of evidence for the deleterious effect of hookworm, but not to change the direction of the differences.

For these main reasons, as well as for others of relatively minor significance, our data represent the total burden of sickness inadequately and disproportionately less adequately for men infected with hookworm than for men in whom the infection was not detected. Our computations and conclusions therefore do not overstate the volume of the burden resulting from hookworm.

Another factor which operates to decrease the contrast in sickness between men with hookworm and men in whom it was not detected, results from the fact that not all men with hookworm were detected. A single examination is insufficient to detect all infections. Tests made by Lieutenant Kantor on men in the hospital at Fort Oglethorpe, Ga., showed that the first examination revealed only 61 per cent. of the infections, detected on five examinations, among men in whom infections were presumably as a rule heavier than in troops from Texas and Oklahoma. The average percentage of infection in Camp Bowie was 12.3 per cent. Many of the stools examined were very lightly infected. Infections in which male worms only are present are not detected by the methods used in stool examination. In addition many of the men of Camp Bowie came from the outer fringes of the hookworm area, and were in the period of life in which the hookworms entering the body in the barefoot days of boyhood are dying of old age. This is estimated at seven years, for males, and ten for female worms. The preponderance of light infections in these troops is therefore to be expected.

In the course of our survey at Camp Bowie there were a number of instances of men negative or positive on one examination for whom the finding was reversed at the second examination. In 14 instances of examination at autopsy, of men previously reported as negative on the stool examination, Major Barber at Camp Jackson, S. C., found 8 or 57 per cent. carrying a light infection, as shown by an average of 9 worms per man, as compared with 85 worms in 4 previously reported positives. While it is hardly probable that either 39 per cent. or 57 per cent. of our negatives at Camp Bowie were even lightly infected, it is certain that an unknown smaller number of them were so infected. These men are included in our negatives, and whatever excess of sickness they may have had, as a result of the undetected infection, reduces the contrast in our findings between the men with hookworm and those in whom we did not detect it.

These considerations, as a whole, make it highly probable, if not certain, that the burden of sickness upon the army, and upon the communities in the hookworm area from which these troops came, which is fostered, if not created, by hookworm, was in excess of that which this investigation has revealed. The authors are fully aware of the inadequacy of the data and of the very complex nature of the problem on which they bear. We have attempted in face of these difficulties to isolate this factor, and to determine its probable potency in so far as the data will permit.

It should be distinctly noted that we fully recognize that hookworm is not the sole and only potent factor involved. Malaria doubtless enters into consideration to some extent in troops from Southeastern Texas, but a large part of the area from which the 36th Division came is relatively free from this plague. There are only 24 cases of active malaria on record for the 22,842 men included in our investigation.

Hookworm is only one element in a vicious circle of interacting factors including among others, lack of sanitation in rural districts, the selective action of the movement of population to cities, the hereditary segregation of classes resulting therefrom, the lack of exposure in childhood to infectious and contagious diseases which results from the isolation of individuals in rural communities, and parasitism by hookworm and malaria, persistent chronic infections. All of these factors cooperate to foster and maintain the higher sick rate which we have here correlated with the infection by hookworm alone. The elimination of this infection presumably tends to reduce this burden of sickness, and the extent of this reduction would in time constitute a rough measure of the volume of burden contributed by hookworm. In individual cases of treatment and cure in the army this reduction is patent, but no quantitative measure of the result in numbers, adequate to afford a test, is available.

PRESENTATION OF THE STATISTICAL DATA.

The statistical data presented are drawn from the various regiments and units comprising the 36th Division. Each organization has been worked out in detail in Table I, with comparative tabulation of pertinent data, those with stools in which ova of hookworm were detected, against those in which ova were not found. The figures on the so-called negative side are taken as the normal upon which computations of percentages have been made.

On the left and right appear respectively, data dealing with men in whose stools hookworm ova were not detected and those in which ova were found. The first two columns on the right of the heavy line contain the total number of men in whom hookworm was detected and the per cent. infected among the whole number examined. These numbers are of necessity larger than those in the next column, for sick data were not obtainable on many of the positives. The total number of men with ova in their stools is in excess of those whose sick and hospital records were available for use.

Corresponding columns on the left and right deal with total number of men with data, total number of men sick, and total number of sick calls and hospital admissions and computation of the same per man in each case. If a man appeared four times on sick call, the number four (4) would be added to the total sick calls, but he would be recorded in the sick column only once. Also if he appeared twice on sick call and once in hospital, his record would appear as two and one in these columns, but only once as sick. Using this method, we have the total number of men who have been ill, distinct from the number of times they have been on sick call or in the hospital.

The last three columns on the right deal with the per cent. of increase or decrease of admissions to hospital, total sick calls, and total number of men sick among the positives, as compared with the so-called negatives. The figures are self-explanatory, if we remember that computations use the negative side as a fair representation of the average normal.

Reports of two typical regiments are given in detail, the 141st Infantry (Table I) and the 143d Infantry (Table II). These show how company and regimental totals were obtained by a study of each individual in each company. Tables III to V deal with the Division as a whole. Table III is a grand summary of all the regiments and the other units, with additional mortality statistics for each unit. The next (Table IV) deals with the incidence of various diseases occurring in positive and so-called negative individuals respectively, and the last (Table V) is a comparison of mortality and morbidity in so-called negatives and positives for hookworm in the Division as a whole.

The facts brought out show definite trends towards important and definite conclusions. These are the more accurate and the more significant by reason of the great mass of data. Not only in individual regiments, companies, or units, but in the Division as a whole, the striking influence of hookworm infection is definitely directed against army efficiency. Thus certain fundamental problems arise:

1. What is the influence of hookworm infection on the incidence of sickness, number of daily sick calls, and the hospital admissions in this Division?
2. What influence has hookworm infection on the chronicity of minor ailments?
3. Does hookworm infection influence the severity of infectious diseases?
4. Does hookworm infection increase susceptibility to disease?
5. What bearing has hookworm infection on mortality?
6. What influence has hookworm infection on army efficiency?

Attention is directed to the tabulated data and conclusions for a consideration of these questions.

MORBIDITY IN THE 141ST INFANTRY.

In the 141st Infantry, a lightly infected regiment with only 11.7 per cent. of hookworm infection, data are recorded on 2,263 men. This does not comprise the entire number examined, but those whose sick and hospital records were obtainable. On the left hand side of the table (Table I) 1,974 men are reported showing no ova in one stool examination. Of these 851 were sick at some time or other, giving 315 admissions to the hospital and 888 appearances on sick call. In this same organization, we have records on 289 positives with 164 sick, 48 hospital admissions and 178 sick calls. The increase of positives over so-called negatives is 31.6 per cent. in men sick, 56.2 per cent. in hospital admissions, and 108.1 per cent. in sick calls. All of the individual companies in the regiment show corresponding gains in one or more of the three columns, gains in disease being

TABLE I.

Incidence of sick call and hospital admission in men with hookworm infection, as compared with those in whom this was not detected, in the 141st Infantry, Camp Bowie, Texas.

Co.	No hookworm detected.						Hookworm positive.										
	Total men.	No. sick.	Times in hospital.		Times on sick call.		Total men with hookworm.	Per cent. infected.	Total men with sick data.	No. sick.	Times in hospital.		Times on sick call.		Per cent. increase or decrease in hospital.	Per cent. increase or decrease on sick call.	Per cent. increase or decrease in number of men sick.
			Total no.	No. per man.	Total no.	Calls per man.					Total no.	No. per man.	Total no.	No. per man.			
A.	195	53	14	0.07	67	0.34	2	1.1	2	1	0	0	1	0.5	−100.0	47.06	84.0
B.	118	55	24	0.2	56	0.47	10	7.8	10	6	0	0	6	0.6	−100.0	27.66	28.7
C.	166	94	55	0.33	86	0.52	10	5.2	10	6	2	0.2	5	0.5	−39.39	− 3.85	5.9
D.	127	62	18	0.14	73	0.57	11	7.3	11	4	2	0.18	7	0.64	28.57	12.28	−25.5
E.	116	51	18	0.16	56	0.48	16	11.8	16	10	4	0.25	8	0.5	56.25	4.17	42.2
F.	116	58	20	0.17	64	0.55	10	7.5	10	8	2	0.2	10	1.0	17.65	81.81	60.0
G.	99	61	23	0.23	56	0.56	8	6.2	8	4	1	0.12	6	0.75	−47.83	33.93	−18.8
H.	83	41	14	0.17	42	0.5	25	17.7	25	16	3	0.12	17	0.68	−29.4	36.0	29.5
I.	116	67	25	0.21	65	0.56	25	12.0	22	15	6	0.27	20	0.91	28.57	62.5	18.0
K.	97	49	10	0.1	57	0.59	9	7.5	9	4	0	0	6	0.67	−100.0	13.56	−12.0
L.	101	64	28	0.28	64	0.63	68	32.8	68	48	15	0.22	45	0.66	−21.43	4.76	11.4
M.	143	55	18	0.12	70	0.49	28	15.2	28	17	8	0.28	23	0.82	133.3	67.35	57.8
Hdq.	193	36	6	0.03	38	0.19	32	12.5	29	2	1	0.03	1	0.03	0.0	−84.21	−63.0
M.G.	127	61	21	0.16	39	0.30	19	12.0	19	14	4	0.21	12	0.63	31.25	110.0	53.4
Sup.	122	34	14	0.11	48	0.39	19	12.7	19	8	0	0	10	0.53	−100.0	35.89	51.1
M.D.	55	10	7	0.13	7	0.13	3	4.5	3	1	0	0	1	0.33	−100.0	153.84	83.3
Total. . . .	1,974	851	315	0.16	888	0.45	295	11.7	289	164	48	0.25	178	0.94	56.25	108.08	31.6

shown in thirty-three of the forty-eight entries on the positive side. Yet various factors come in to change the balance here and there without materially affecting the direction of the difference in the regiment as a whole.

In Company A, only two positives are recorded with no admissions to hospital and only one sick call. Here the so-called negatives have a gain of 100 per cent. in hospital admissions. Yet obviously, with only two positives against 195 with no ova in stools, no great significance can be placed on this particular case. Doubtless individual hookworm infections in this company were of light intensity, for its men came from lightly infected districts. Records show that 65 per cent. were recruited from El Paso County, Texas, where the percentage of men of the Division from this county found to be infected in 404 examinations was only 4.4 per cent. Of the other counties from which the men of this company were drawn approximately 90 per cent. are lightly infected regions.

The increase of severity in disease among these positive men is indicated by the higher percentage of hospital admissions among them, as compared with the percentage of men sick, the whole regiment giving 31.6 per cent. men sick above the normal so-called negatives, while the hospital admissions show 56.25 per cent. increase. The difference, 24.65 per cent., shows that there is an actual increase in severity of disease among men positive for hookworm.

The same principle applies in considering chronicity of minor ailments, for where percentage of sick calls exceed percentage of men sick, minor ailments are shown to have caused more sick calls and are more chronic. The sick call shows 108.1 per cent. increase in positives above normal, as represented by so-called negatives, and is 76.5 per cent. higher than the increase in men sick. That is, there are more chronic illnesses among men carrying the infection than among the men in whom hookworm was not detected.

Morbidity in the 143d infantry.

This was a heavily infected regiment having no less than 30 per cent. infections. Consideration of Table II, containing data on the 143d Infantry shows the same direction of difference, as that in the 141st Infantry. Here the general hookworm infection is heavier throughout the regiment. In companies C, D, and E, the positives exceed in number those in whose stool no ova were detected. Each of these companies shows a definite increase in the percentage of men

TABLE II.

Incidence of sick call and hospital admission in men with hookworm infections, as compared with those in whom this was not detected, in 143rd Infantry, Camp Bowie, Texas.

Co.	No hookworm detected.						Hookworm positive.										
	Total men.	No. sick.	Times in hospital.		Times on sick call.		Total men with hookworm.	Per cent. infected.	Total men with sick data.	No. sick.	Times in hospital.		Times on sick call.		Per cent. increase or decrease in Hosp.	Per cent. increase or decrease on sick call.	Per cent. increase or decrease in number of men sick.
			Total No.	No. per man.	Total No.	Calls per man.					Total No.	No. per man.	Total No.	No. per man.			
A	130	70	47	0.35	93	0.71	33	20.7	33	17	12	0.36	32	0.97	2.86	36.6	– 4.3
B	130	76	78	0.6	524	4.03	67	33.9	64	39	42	0.66	243	3.8	10.0	– 5.71	4.2
C	56	34	22	0.41	62	1.15	131	68.0	131	88	57	0.43	177	1.35	4.88	21.7	10.7
D	73	54	30	0.41	582	7.97	122	62.9	122	115	67	0.55	1,290	10.6	34.15	33.0	27.4
E	87	36	39	0.45	173	1.99	94	54.3	94	46	43	0.46	230	2.45	2.22	23.11	18.3
F	98	85	40	0.41	155	1.58	83	46.0	83	80	29	0.35	149	1.8	–14.63	13.92	11.1
G	141	121	61	0.43	442	3.13	40	21.8	39	32	21	0.54	127	3.26	25.58	4.15	– 4.4
H	152	131	51	0.33	421	2.77	54	25.7	54	45	25	0.46	156	2.89	39.39	4.33	– 3.3
I	182	165	82	0.45	596	3.27	24	11.9	24	18	5	0.21	81	3.37	–53.3	3.06	–17.3
K	118	59	38	0.32	462	3.91	86	43.9	86	58	39	0.45	293	3.4	40.62	–13.04	34.9
L	176	123	83	0.47	306	1.73	28	13.4	28	16	12	0.43	47	1.68	– 8.51	– 2.89	–18.2
M	183	90	88	0.48	182	0.99	17	8.4	17	4	5	0.3	0	0	–37.5	–100	–52.1
Hdq.	195	142	60	0.31	366	1.88	44	17.7	42	33	4	0.1	94	2.24	–67.74	19.15	7.9
M.G.	124	96	47	0.38	176	1.42	30	20.4	30	20	15	0.5	46	1.53	34.27	7.75	–13.9
Sup.	138	28	26	0.19	24	0.17	31	22.3	31	12	11	0.36	45	1.45	89.47	753.0	90.8
M.D.	34	14	13	0.38	24	0.7	11	20.8	10	4	1	0.1	6	0.6	–73.7	–14.3	– 2.9
Total	2,017	1,324	805	0.4	4,588	2.27	895	30.0	888	627	388	0.44	3,016	3.4	10.0	49.9	7.6

sick, of admission into the hospital, and number of sick calls in infected men as compared with the non-infected. As in the 141st Infantry, as a rule, the companies with the smaller number of positive infections show a smaller percentage of gain. The positive hookworm cases in companies C, D, E, and K, have an increase in men sick of 10.7 per cent., 27.4 per cent., 18.3 per cent., and 34.9 per cent. above those who showed no hookworm ova in one stool examination. Throughout the left or negative half of the table, we find also, a general increase in the total men sick, in hospital admissions, and in sick calls above the general level in the Division. It is reasonable to conclude that some hookworm cases in this regiment remained undiscovered by a single examination of stools for ova, and may have been potent in affecting the morbidity of the so-called negatives.

Company C, out of which 131 positives were recorded, with 56 men in whom no ova were discovered, comes largely from a particularly heavily infected region of Texas. Over 75 per cent. of the number were recruited from the following eight counties: Hardin, Orange, Jasper, Smith, Angelina, Sabine, Newton, and Tyler. In these, the rate of infection, based on combined records for Texas in the Southern Department, varied from 25.9 to 63.0 per cent., the lowest in Hardin and the highest in Tyler County. With over three fourths of the company coming from such hotbeds of hookworm infection, it is reasonable to conclude that a great many of the so-called negatives would have proved positive on further examination of the stools. Hence, any conclusions drawn from the hookworm side of the table are within the facts. No doubt figures on this side are much lower than the facts would really show, if all positives had been detected, yet in the regiment as a whole, sickness predominated much more among hookworm victims than those in whom it was not detected. The regiment had a total of 2,017 men, whose stools were reported negative, and 888 with sick data, who were positive with a total gain of 7.6 per cent. in men sick, 10 per cent. in hospital admissions and 49.9 per cent. on sick call. The greater severity of diseases in positives than in so-called negatives is indicated by the facts that with an increase of only 7.6 per cent. in men sick among positives, there is among them, an increase of 10 per cent. in hospital admissions. Greater chronicity of disease in positives as compared with negatives, so-called, is shown by the fact that with an increase of 7.6 per cent. in men sick, there is an increase of 49.9 per cent. in sick calls.

TABLE III.

Incidence of sick call and hospital admission in men with hookworm, as compared with those in whom hookworm was not detected.
Summary of organizations—36th division, at Camp Bowie, Texas, October 1, 1917 to April 15, 1918.

Organization.	No hookworm detected								Hookworm positive.										
			Times in hospital.		Times on sick call.		Mortality.*						Times in hospital.		Times on sick call.		Per cent. increase or decrease		
	Total men.	No. sick.	Total no.	No. per man.	Total no.	No per. man.	No. dead.	Per cent. dead.	Total men with hookworm.	Per cent. infected.	Total men with sick data.	No. sick.	Total no.	No. per man.	Total no.	No. per man.	In hosp.	on sick call.	In no. of men sick.
141 Inf.	1,974	851	315	0.16	888	0.45	36	1.4	295	11.7	289	164	48	0.25	176	0.94	56.25	108.1	31.6
142 Inf.	2,559	1,347	417	0.16	2,605	1.02	22	0.7	295	9.8	278	150	52	0.19	300	1.08	18.7	5.9	2.5
143 Inf.	2,017	1,324	805	0.40	4,588	2.27	89	2.8	895	30.0	888	627	388	0.44	3,016	3.4	10.0	49.9	7.6
144 Inf.	2,602	1,000	450	0.17	1,107	0.42	46	1.4	368	12.1	363	172	96	0.26	195	0.54	52.9	28.6	23.3
131 F.A.	1,238	833	124	0.10	1,508	1.22	13	0.9	143	10.5	142	112	51	0.36	268	1.89	259.0	54.9	78.9
132 F.A.	1,100	270	37	0.03	278	0.25	4	0.3	144	11.3	135	37	3	0.02	42	0.31	−33.3	24.0	−11.4
133 F.A.	1,293	480	95	0.07	614	0.47	20	1.3	163	11.2	162	78	15	0.09	113	0.7	28.6	48.9	29.7
131 M.G.Bn.	349	171	116	0.33	302	0.86	9	2.3	37	10.1	36	13	16	0.44	16	0.44	33.3	−48.8	−26.1
132 M.G.Bn.	639	239	59	0.09	288	0.45	2	0.3	63	9.1	63	23	16	0.25	43	0.68	177.8	51.1	− 2.4
133 M.G.Bn.	513	318	133	0.26	496	0.97	7	1.0	140	21.2	132	88	46	0.35	146	1.11	34.6	14.4	7.5
111 Eng.	1,389	633	343	0.25	1,568	1.13	18	1.2	132	8.8	132	89	82	0.62	203	1.54	148.00	36.3	48.0
111 Am.Tr.	796	238	63	0.08	213	0.27	11	1.1	56	6.4	53	23	12	0.23	32	0.6	191.1	122.2	45.1
111 F.S.Bn.	382	212	96	0.25	1,079	2.82	3	0.6	40	9.6	39	25	11	0.28	153	3.92	12.0	39.0	15.5
111 Sup.Tr.	398	194	45	0.11	251	0.63	5	1.1	59	13.4	59	34	9	0.15	50	0.85	36.3	34.9	18.2
111 M.P.	177	115	47	0.26	202	1.14	1	0.2	63	17.8	63	55	18	0.28	95	1.51	7.7	32.4	34.4
111 San.Tr.	796	329	133	0.17	409	0.51	3	0.3	56	7.2	55	27	17	0.31	27	0.49	82.3	− 3.9	18.8
Miscellaneous	1,606	537	143	0.09	1,098	0.68	18	0.8	132	7.2	125	50	28	0.22	116	0.93	144.4	36.8	19.6
Grand total	19,828	9,091	3,421	0.17	17,494	0.88	307	1.2	3,081	13.5	3,014	1,767	908	0.3	4,993	1.66	76.5	88.6	27.9

* Per cent. mortality is based upon total men on roster.

MORBIDITY IN THE 36TH DIVISION AS A WHOLE.

Data from the Division as a whole show in even a more convincing way the trend of affairs. In Table III all regimental and unit-totals have been added together with a calculation of resulting gain or loss in hospital admissions, sick calls and men sick. It represents 22,842 men who have had one stool examined for hookworm ova, and concerning whose health the sick cards and hospital records have been consulted. It includes a larger proportion of negatives from lightly infected organizations, from lightly infected regions, than in the case of the 141st and 143d Infantry which we have discussed above. The masking effect of undetected positives among the so-called negatives is therefore less in evidence in the Division, as a whole, than in the more heavily infected portions thereof. In the Division there were 3,014 positives and 19,828 men showing no ova on the stool examination. In other words, of men with sick data, 13.5 per cent. showed hookworm ova on one examination of the stool. Among these 13.5 per cent. positives, there was an increase of 27.9 per cent. in men sick,—over one fourth more, than in the so-called negatives. Hospital admissions are 76.5 per cent. higher in positives or over three fourths above the normal. Sick calls show 88.6 per cent. increase among hookworm hosts, which almost doubles the ratio among so-called negatives.

As in the 141st and 143d Infantry, the Division as a whole shows greater severity of disease among the positive hookworm cases for, whereas the increase in the positives over the so-called negatives in number of men sick is only 27.9 per cent. the increase in hospital admissions is 76.5 per cent. That is, the relative greater gain in admissions to hospital indicates an increase in severity of disease among positive hookworm cases, as compared with the so-called negatives. Likewise chronicity of disease is greater in positives, for the number of sick calls shows a gain of 88.6 per cent., while the number of men sick shows a gain of only 27.9 per cent. in the positives. The much greater gain in number of sick calls, per man, among positives, as compared with the so-called negatives, indicates that in the former diseases are much more chronic in character. This coincides with the data recorded on the several regiments and units of the division.

It is obvious that in a summary of this extent, minor variations in the direction of difference among companies or regiments and units, become, by preponderance in one direction, less and less significant. Occasional divergences are bound to exist among sick and hospital records, as a result of the varying thoroughness of work done by the regimental surgeons and their staff. Yet in the totals, these differences become submerged by the weight of accurate records. Hence, the striking figures which appear in the grand summary of the whole division only repeat more forcibly the trend of the data in its various units. That is, sickness, whether mild or severe, acute or chronic, is increased among the men who are weakened in general health by hookworm infection, as shown by the general trend in the detailed data and by the final totals. The *direction of the differences* in morbidity detected between men with hookwork infection and those presumably without it, are of prime significance; the amplitude of these differences as here computed, is subject to varying degrees of error.

In October, 1916, the prevalence of illness and disease among certain organizations on the Mexican border, aroused the army surgeons to a preliminary investigation by Foster and Sinclair (1916) to ascertain its cause. At Nogales, Arizona, among 1,259 men, chosen at random from organizations composing a brigade of Alabama National Guard, 39.9 per cent. showed hookworm ova on a single stool examination. From October 24, 1916, to February 24, 1917, a period of four months, 84 per cent. of the 1st Alabama Infantry had been on sick call one or more times. During this same period 85.7 per cent. of the 4th Alabama Infantry were on sick call. In other words, the investigators found a very high incidence of sickness in a Brigade where hookworm was so prevalent. The present survey leads in the same direction, to the same general results, although it is much more comprehensive in its scope and significance, and involves larger numbers.

Incidence of different diseases.

The incidence of different diseases among men with hookworm ova in their stools shows, in the main, no striking evidence of heightened susceptibility to particular diseases (Table IV). The diseases that show a small percentage of increased incidence are tonsillitis, laryngitis, bronchitis, pneumonia, certain skin diseases, neurological affections, and of other miscellaneous acute infections, anthrax, erysipelas, cellulitis, peritonitis, chickenpox, septicaemia, and measles. The others show a percentage decrease, varying from minus 5.7 to minus 100. These latter are of less frequency and less importance in army economy.

TABLE IV.

Incidence of different diseases in men with hookworm and in those in whom this was not detected, in the 36th Division, Camp Bowie, Texas.

Diseases.	No hookworm detected.		Hookworm positive.		Per cent. increase or decrease among hookworm positives.
	Number.	Per cent. of disease.	Number.	Per cent. of disease.	
Respiratory:					
Asthma	12	0.12	1	0.01	− 91.6
Bronchitis	1,254	12.70	289	17.30	+ 36.2
Empyema	24	0.24	2	0.12	− 50.0
Influenza	1,130	11.40	171	10.20	− 10.5
Laryngitis	60	0.60	17	1.02	+ 70.0
Pleurisy	92	0.93	12	0.72	− 22.5
Pneumonia	739	7.40	136	8.10	+ 9.5
Pharyngitis	178	1.80	26	1.50	− 16.7
Rhinitis	119	1.20	9	0.54	− 55.0
Tuberculosis	37	0.39	2	0.12	− 69.2
Total	3,645	36.90	665	39.90	− 8.1
Gastro-intestinal	382	3.80	59	3.50	− 7.9
Constitutional, Diabetes, etc.	449	4.50	76	4.50	0
Cardio-vascular	25	0.25	2	0.12	− 52.0
Miscellaneous: Alcoholism, Cholecystitis, Hemorrhoids, etc.	705	7.10	68	4.10	− 42.3
Genito-urinary	460	4.60	61	4.00	− 13.0
Skin Diseases	28	0.28	6	0.36	+ 28.9
Neurological	34	0.34	25	1.50	+ 77.3
Special Senses Otitis Media, etc.	309	3.10	51	3.12	+ 0.6
Acute infections:					
Adenitis	24	0.24	2	0.12	− 50.0
Appendicitis	63	0.63	5	0.36	− 42.8
Anthrax	5	0.05	0	0	+ 100.0
Abscess	111	1.12	11	0.70	− 37.8
Arthritis	19	0.19	1	0.01	− 94.7
Cellulitis	54	0.55	10	0.60	+ 8.3
Cerebro-spinal meningitis	34	0.34	3	0.18	− 47.0
Chicken pox	6	0.06	2	0.12	+ 100.0
Diphtheria	6	0.06	0	0	− 100.0
Erysipelas	9	0.09	4	0.24	+ 166.7
Measles	612	6.20	130	7.80	+ 25.8
Mumps	2,095	21.20	333	20.00	− 5.7
Peritonitis	1	0.01	2	0.12	+1100.0
Rheumatism	177	1.70	27	1.60	− 5.9
Scarlet fever	14	0.40	2	0.12	− 70.0
Septicaemia	4	0.04	2	0.12	+ 200.0
Small pox	2	0.02	0	0	− 100.0
Tonsillitis	594	6.03	118	7.08	+ 17.4
Typhoid fever	3	0.03	1	0.01	− 66.7
Total	3,829	38.80	653	39.00	− 0.5

The classifications given are those found in the sick and hospital records. It is unfortunate that an examination of individual histories was not feasible. Unfortunately this survey, by its very volume, compelled the work to be extensive rather than intensive. Yet, as much emphasis was placed on the latter phase, as time and conditions would permit.

General discussion and comments.

This survey for the detection of a possible relation between morbidity, mortality, and infection by hookworm in men comprising the 36th Division, at Camp Bowie, Texas, covers over 20,000 men through a period of nearly seven months, from October 1917 to April 1918. Those engaged in the investigation met with many difficulties and perplexities which tended to impair the accuracy of the work. No claim is made that the statistics compiled in this report are without errors. In fact there are some instances where reports available from regimental infirmaries were imperfect. In such instances an effort has been made to re-compile the data, correct the errors, or throw out altogether patent imperfections. In every instance where a choice has been open, we have thrown the weight of the evidence against hookworm, that is, to minimize, rather than exaggerate its importance. Hence conclusions reached have been based upon data conservatively compiled, where there was an option.

The greatest source of error doubtless arises from the necessity in each case of limiting the diagnosis of negative hookworm to an examination of one stool. As shown above this results in many light infections escaping detection and appearing in consequence in the list of negatives. For this reason we have used the phrase, "so-called negatives." The masking effect of the undetected positives cannot be measured from available data. We know neither their numbers nor the incidence of disease and death among them, nor have we any evidence as to whether or not light infections affect morbidity and mortality. The fact that total morbidity and mortality are higher in organizations with over 10 per cent. infection than they are in regiments with less than 10 per cent., may be due to the larger relative numbers of undetected positives in the former, or to heavier individual infections or quite probably to both factors in combination.

This investigation deals with many men from the margins of the hookworm area, in the period of life when the infections are dying out, and with a group of men from which severe cases of hookworm disease have presumably been weeded out by medical examination at the time of enlistment or draft. The 36th Division was composed of National Guard troops supplemented by the earlier increments of the draft. Clinicians, treating men reported positive as a result of one examination, state that less than 10 per cent. of the men showed any noticeable clinical signs of the disease. The men in the great majority of the cases are carriers in light chronic phases of the infection and would not ordinarily be regarded as diseased by hookworm.

In the Division as a whole, and in most of the regiments and individual companies, the number of men becoming sick for any reason was greater among the hookworm carriers than in so-called negatives, being 27.9 per cent. of the total. Likewise, the tables show that the number of daily sick calls and admissions to hospitals were increased 88.6 per cent. and 76.5 per cent. respectively. These minor disturbances of health which cause a soldier to answer sick call, were thus more chronic among positives, inasmuch as the average calls per man were greater among them than the so-called negatives. The instances of men reporting sick to escape duty would presumably apply equally to positives and negatives, without much disturbing the relative values. In addition the greater number of hospital admissions per man, among positives, as compared with negatives, indicates that diseases were more severe in the former than in the latter. It is quite generally recognized that tuberculosis, malaria, and other chronic illnesses prove very stubborn or almost intractable to treatment in patients who have the additional handicap of hookworm infection. In fact, the intestinal tract must be cleared of parasites before the vital resources of the patient can be fully mobilized to arrest or obliterate disease efficiently. The facts in our data reveal the additional burden that was placed upon the Medical Corps of this Division by hookworm infection.

The available data here do not show very convincingly whether or not positives are more susceptible to the incidence of particular diseases than negatives. Yet it is of some value to note that respiratory diseases such as tonsillitis, laryngitis, bronchitis, and pneumonia, as well as measles, all of which exist very extensively in the army, were of greater incidence in this camp in men with hookworm than in men without. That the severity of disease is higher in positives than in negatives is shown by the 76.5 per cent. increase in hospital admissions, and by the fact that ten organizations having more than 10 per cent. hookworm infection, had a mortality rate of 1.5 per cent., 87 per cent. above that of seven others with lighter infections, averaging 8 per cent.

Wherever data in our records are large enough to reduce ordinary sources of error, whether we consider incidence, type in some instances, severity, chronicity, or mortality of disease, the frequency is as a rule greater in men with hookworm than in so-called negatives. That is, the direction of the difference in these particulars throws the burden of disease and its consequences onto men known to have hookworm infection.

All in all, those infections which are most common in army life and the easiest to diagnose, show a considerable increase among the hookworm hosts. Of the respiratory diseases, tonsillitis shows an increase of 17.4 per cent., laryngitis 70 per cent., bronchitis 36.2 per cent., pneumonia 9.5 per cent. and of the exanthemata, measles is 25.8 per cent. above the normal.

A survey was made by Cole and Siler (1917) of the 1st Mississippi Infantry and the 1st Alabama Cavalry, in which 1,710 recruits were examined for hookworm. Among 750 hookworm positives 136 cases of morbilli developed, while among 967 negatives only 63 cases of this disease occurred. In other words, there was an increase of 157.1 per cent. in measles among hookworm men. Complications, secondary to this eruptive disease, were 14 per cent. in positives and 8 per cent. in the so-called negatives, or an increase of 75 per cent. for those having the parasite. The same writers have shown that pneumonia was most prevalent among the men coming from the rural communities of the South. It is in these districts too, that hookworm infests the greatest percentage of the population.

It is obvious that immunity to measles is conferred only by a previous attack of the disease, and that the opportunities for this infection are greater in urban than in rural life. The figures cited from Cole and Siler are presumably to be explained by the isolation from infection by measles, in the troops of rural origin, and by the heavier incidence of hookworm consequent upon rural sanitation. In the absence of knowledge as to the number of measles susceptibles in the two groups of hookworm and non-hookworm men we find no evidence here that hookworm increased the susceptibility of its victims to measles infection. It may be expected to have increased the *severity* of the attacks among its victims so that they all came to observation and record, while in men without hookworm some of the attacks may not have been so heavy, because of the absence of the infection.

It is highly probable that the incidence of other infectious and contagious diseases is not increased among hookworm victims by reason of their infection by this parasite. These diseases may, however, run a longer and severer course by reason of depleted resistance resultant from the toxaemia caused by this parasite. It may even be that an apparent increase in the *incidence* of diseases, such as those caused by light infections of the respiratory organs, may result from the fact that the increased severity brings them to the level of observation and of record more often in men with hookworm than in those without this infection. Men in both groups may be infected, but only the hookworm victim be "sick" enough to require and receive medical care, and thus be entered on the record, while the equally infected non-hookworm man recovers quickly and escapes medical care and record.

In a hookworm survey at Camp Sevier among the healthy soldiers, 18.6 per cent. were infected with hookworm. Among fatal cases of broncho-pneumonia, 50 per cent. were positive for hookworm, while among those that recovered, only 17 per cent. had the parasite. Since the percentage of hookworm among the recoveries was even less than the percentage of this infection among healthy men of this command, it is quite reasonable to regard hookworm as having a powerful influence against recovery from broncho-pneumonia.

Reports from Camp Jackson and Camp Wheeler state that in the former hookworm infection was present in about 30 per cent. of the command, and in the latter "many men suffered from anemia of hookworm." These camps, made up of Southern troops, show a similar prevalence and deleterious effects of hookworm infection that are brought out in this detailed survey of the 36th Division at Camp Bowie.

It is possible that the passage of hookworm larvae through the pulmonary capillaries into the alveoli during the period of invasion, renders the broncho-pulmonary tract more susceptible to disease. It is rather hazardous in the absence of controlled experiments, to venture a conclusion from statistical study whether or not such a lowering of resistance does occur. Should any pulmonary sensitization be present, it would of necessity be incurred during the "ground itch and snuffles" period. Practically all of these positives have long since passed beyond the stage of invasion and come under the classification of chronic carriers. Hence they might have developed a general lowering of resistance as well as this hypothetical local one. Whether one or both of these factors are at work requires extended clinical research. The statistics show that pneumonia is increased 9.5 per cent., bronchitis 36.2 per cent., and laryngitis 70.0 per cent. in those men with hookworm.

The incidence of tuberculosis shows a decrease of 69.2 per cent. among men harboring hookworm. At first glance it is difficult to understand why this disease should be so infrequent in these men. Yet this survey was conducted in the early period of mobilization, when the rigorous army life had had little time to affect those with less endurance. Any active or severe chronic tuberculosis would have been eliminated by the draft boards, at time of enlistment. The clinical picture of hookworm infection, plus active or severe pulmonary tuberculosis, would scarcely be overlooked in even the most cursory examinations and would result in rejection of men with the combined infection. The same thing would occur in most of the chronic constitutional diseases.

MORTALITY AND HOOKWORM IN THE 36TH DIVISION.

The tabulation of mortality, morbidity, and hookworm infection in the 36th Division from October 1, 1917, to April 1, 1918, shown in Table V, gives the complete data in parallel columns. The figures show no uniform increase in mortality among the regiments with the higher percentages of infection. In the 143d Infantry 30 per cent. of the men had hookworm ova in the stool examination, with 67.2 per cent. sick and a mortality of 2.8 per cent. This is twice, or more, the death rate of any other unit except the 131 M.G. Battallion and 2.3 times the average death rate of the whole division in this four month period. The 133d M.G. Battallion, with 21.2 per cent. hookworm infection and 62.9 per cent. of men sick, had a mortality of only one per cent. It is difficult to say why this heavily infected organization does not show a correspondingly high death rate, beyond the fact that it is a small organization. The former regiment had about five times as many men on roster, and over six times as many men with hookworm ova detected in their stools. It is the heaviest infected organization, and has the highest death rate in the Division.

There are ten of these organizations with 14,306 men examined which have a total hookworm infection of over 10 per cent. and seven with 9,118 men examined with the infection below this figure. If the morbidity and mortality are compared in the two groups there is a definite preponderance of both sickness and death in the more highly infected group, as shown in Table VI.

TABLE V.

Mortality, morbidity and hookworm infection in the 36th Division, at Camp Bowie, Texas, from October 1, 1917 to April 1, 1918.

Organization.	Total men on roster.	Mortality.		Total men with data.	Total men sick.		Total men exam. for hookworm.	Total men with hookworm.	
		No. dead.	Per cent.*		No.	Per cent.†		No.	Per cent.‡
141 Inf.	2,589	36	1.4	2,263	1,015	44.9	2,521	295	11.7
142 Inf.	3,189	22	0.7	2,837	1,497	52.8	3,010	295	9.8
143 Inf.	3,165	89	2.8	2,905	1,951	67.2	2,983	895	30.0
144 Inf.	3,306	46	1.4	2,965	1,172	39.5	3,032	368	12.1
131 F.A.	1,380	13	0.9	1,380	945	68.5	1,357	143	10.5
132 F.A.	1,449	4	0.3	1,235	307	24.9	1,266	144	11.3
133 F.A.	1,571	20	1.3	1,455	558	38.3	1,452	163	11.2
131 M.G.Bn.	387	9	2.3	385	184	47.8	365	37	10.1
132 M.G.Bn.	721	2	0.3	702	262	37.3	692	63	8.8
133 M.G.Bn.	697	7	1.0	645	406	62.9	658	140	21.2
111 Eng.	1,517	18	1.2	1,521	722	47.5	1,495	132	8.8
111 Am.Tr.	977	11	1.1	849	261	30.7	868	56	6.4
111 F.Sig.Bn.	461	3	0.6	421	237	56.3	446	40	9.6
111 Sup.Tr.	465	5	1.1	457	228	49.8	440	59	13.4
111 M.P.	434	1	0.2	240	170	70.8	232	63	17.8
111 San.Tr.	923	3	0.3	851	356	41.8	772	56	7.2
Miscellaneous	2,298	18	0.8	1,731	587	33.9	1,835	132	7.2
Summary	25,529	307	1.2	22,842	10,858	47.5	23,424	3,081	13.5

* Per cent. mortality is based upon total men on roster.

† Per cent. of men sick is based on total men with data.

‡ Per cent. of men with hookworm is based upon the total number of men examined.

TABLE VI.

Comparison of morbidity, total mortality, and that from pneumonia in organizations of the 36th Division with more or less than 10 per cent. infection by hookworm.

	Above 10%.	Below 10%.
Total number of men examined	14,306	9,118
Total men with data	13,930	8,912
Total men on roster	15,443	10,086
Average hookworm infection	16.1%	8.5%
Morbidity (total men sick)	69.2%	44.0%
Mortality	1.5%	0.8%
Mortality from pneumonias	1.3%	0.6%

It appears from this compilation that men in the more heavily infected organizations—above 10 per cent.—(average 16.1 per cent.), have a morbidity (total men sick) of 69.2 per cent., as compared with 44 per cent. in organizations with less than 10 per cent. hookworm infection (average 8.5 per cent.). An increase of 89 per cent. in hookworm infection (from 8.5 to 16.1 per cent.) is accompanied by a 57 per cent. increase in morbidity (from 44 to 69.2 per cent.). In like manner the total mortality rises from a ratio of 0.8 per cent. to 1.5 per cent. with an increase of hookworm infection from 8.5 to 16.1 per cent. An increase of 89 per cent. in the incidence of hookworm is accompanied by an increase of 87.5 per cent. in the total mortality. The total deaths from pneumonia in organizations with 8.5 per cent. hookworm were 64 or 0.6 per cent. In those organizations with 16.1 per cent. hookworm they were 204 or 1.3 per cent. An increase of 89 per cent. in hookworm infections is paralleled by an increase of 117 per cent. in deaths from pneumonias.

TABLE VII.

Comparison of mortality from pneumonias in the 143d Infantry, and the remainder of troops at Camp Bowie.

Number of men on roster.		Per cent. of men examined, infected with hookworm.	Deaths from pneumonias.	Per cent. mortality for pneumonias.	Deaths from all causes.	Total deaths.	Per cent. total mortality.
143d Infantry..........	3,165	30.0	81	2.6	89	170	5.4
Remainder at Camp Bowie.............	22,364	10.7	187	0.8	307	494	2.2

The most striking contrast appears when the 143d Infantry is compared with the rest of the Division, in the matter of rate of mortality from pneumonias. This regiment with 2,589 men on its rosters, at the time of our hookworm survey had 81 deaths from pneumonias. With 10.1 per cent. of the men of the Division on its rosters, it had 30.2 per cent. of the deaths from pneumonias. The mortality rate from these diseases in this regiment with 30 per cent. hookworm was 2.6 per cent., as compared with 0.8 per cent. in the rest of the Division with 10.7 per cent. hookworm, an increase in the rate, of 325 per cent. A 284 per cent. increase in the 143d Infantry in the rate of infection by hookworm above that in the rest of the Division, was accompanied by a 325 per cent. increase in the mortality from pneumonias in the regiment. The following summary presents the data utilized in arriving at these conclusions (Table VIII).

TABLE VIII.

Morbidity and mortality from pneumonia in Army camps, six month's period, September 29, 1917, to March 29, 1918, and hookworm infection.

Camp.	Army.	Annual rate per 1000.		Hookworm.					
				Camp.			Base hospital.		
		Morbidity rate.	Mortality rate.	Per cent. infection.	Positive.	No. of men examined.	Per cent. infection.	Positive.	No. of men examined.
Bowie.......	N.G.	96	20.0	12.3	2,921	23,659	—	—	—
Wheeler......	N.A.	95	23.6	76.4	873	1,143	25.4	63	248
Travis.......	N.A.	78	10.6	10.1	8,657	85,497	4.7	426	9,293
Pike.........	N.A.	63	24.9	2.8	31	1,095	10.7	68	637
Cody........	N.G.	52	9.7	—	—	—	2.2	3	135
Beauregard...	N.G.	42	15.0	10.5	3,304	31,433	9.0	51	568
Taylor.......	N.A.	37	5.4	2.9	962	33,337	9.3	193	2,061
Sevier.......	N.G.	36	11.5	21.0	6,065	28,812	18.0	1,964	10,922
Jackson......	N.A.	36	10.7	18.6	1,887	10,115	21.6	6,667	30,887
Doniphan....	N.G.	33	9.0	—	—	—	—	—	—
Dodge.......	N.A.	29	5.3	4.2	167	4,024	.5	2	422
Funston.....	N.A.	24	10.5	1.6	6	371	—	—	—
Kearney.....	N.G.	24	4.4	1.1	10	871	—	—	7
Lee..........	N.A.	24	5.5	5.0	855	17,229	—	—	—
Shelby.......	N.G.	21	4.7	26.5	344	1,300	9.8	176	1,801
Meade.......	N.A.	18	2.6	—	—	—	10.3	27	262
Logan.......	N.G.	16	1.0	2.4	114	4,807	7.4	559	7,539
Gordon......	N.A.	15	5.3	5.6	268	4,743	5.7	33	576
Sherman.....	N.A.	15	2.5	0.5	20	4,175	6.0	27	446
Upton.......	N.A.	15	3.6	—	—	325	—	—	3
Grant.......	N.A.	14	1.5	—	—	—	1.9	54	2,764
Lewis.......	N.A.	11	1.5	1.9	61	3,157	—	—	—
Devens......	N.A.	9.8	2.0	—	—	—	15.2	835	5,479
McClellan....	N.G.	9.6	1.1	4.9	24	491	18.9	180	955
Sheridan.....	N.G.	9.3	1.7	2.8	48	1,712	4.7	61	1,286
Wadsworth...	N.G.	9.1	1.1	12.9	2,302	17,795	15.2	2,908	19,059
Dix.........	N.A.	8.0	1.5	4.1	234	5,661	16.0	4	25
Custer.......	N.A.	7.0	1.5	1.2	43	3,749	4.3	9	209
Hancock.....	N.G.	6.7	1.1	39.9	2,991	7,502	34.3	394	1,150
Totals.....	......	......	......	11.0	32,187	293,003	15.2	14,704	96,734

Comparison with other camps.

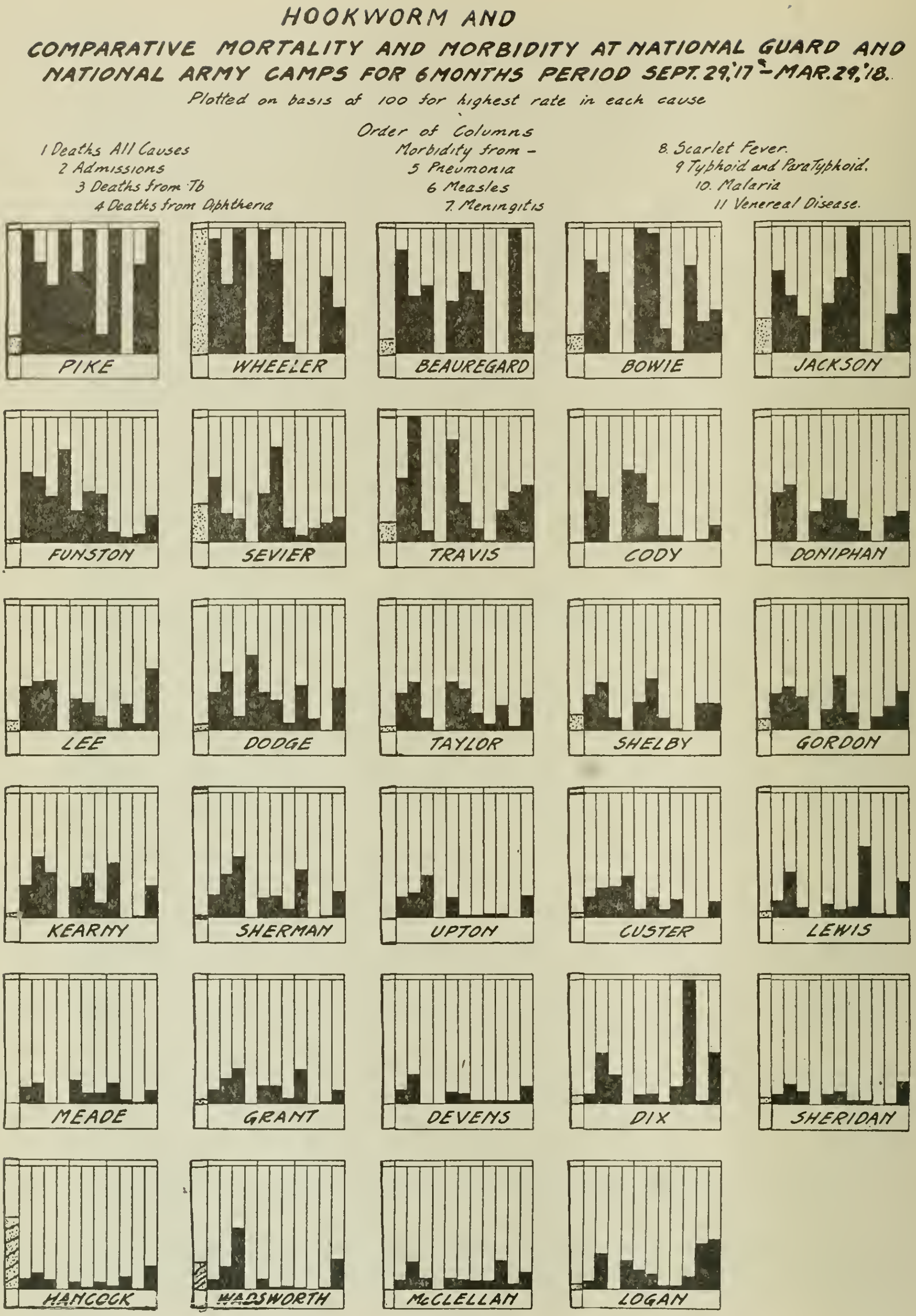
In a comprehensive summary of the conditions of health in troops of the National Guard and National Army for the six months from September 29, 1917 to March 29, 1918, by Col. V. C. Vaughan, M.C., and Captain G. T. Palmer, S.C., there appears a graphic presentation of the comparative mortality and morbidity in 29 camps, which we here reproduce (Fig. 1), with the addition of hookworm infection in each. In this there are plotted the total deaths, hospital admissions, and deaths from tuberculosis, diphtheria, and morbidity from pneumonia, measles, meningitis, scarlet fever, typhoid and paratyphoid, malaria, and venereal disease.

The most striking feature of the ensemble is the greater incidence of disease and death in camps with Southern troops, than in those with Northern. This is shown especially by the higher levels attained by the total death rate, by hospital admissions, and morbidity by pneumonia and measles. The authors note that negroes and Southern whites were more susceptible to pneumonia, than Northern whites, and then state the following: ''It is an opinion generally held by medical officers in Southern camps that hookworm disease and chronic malarial infection increases susceptibility to the acute respiratory diseases. The information we have on these points is not as full as desirable.''

From data compiled for the parasitological section of the proposed Medical History of the War, we are now able to supply data, showing the relative percentages of infection by hookworm in the camps with which these authors were dealing, with some exceptions. Among Northern troops little or no hookworm would be expected. Some enters with migrants from the South and with immigrants from the South of Europe and elsewhere from the hookworm area. Such data as are available in the statistics in this field indicate less than 1 per cent., a merely nominal infection among men enlisting from the North.

We have added in these graphs in a column at the left of the graph for each camp, the percentage of infection by hookworm, plotted on the basis of 100 for the highest rate, as reported to the Surgeon General's Office. The reports of examinations of patients in base hospitals are used in certain instances where they are more representative, than the percentage reported for the camp, as in the case of Camp Pike. A full list of the number of men examined, the number of positives and percentages of infection in camp surveys and hospital patients, wherever these data are available will be found in Table VIII. It is to be regretted that not all camps with Southern troops are represented, and that the data from some are inadequate.

Furthermore the data of Colonel Vaughan's table pertain to troops in camp at a period of about three to six months or even more in some cases, earlier, than the period of hookworm surveys in some



HOOKWORM AND COMPARATIVE MORTALITY AND MORBIDITY AT NATIONAL GUARD AND NATIONAL ARMY CAMPS FOR 6 MONTHS PERIOD SEPT. 29, '17 – MAR. 29, '18.

Plotted on basis of 100 for highest rate in each cause

FIG. 1.

instances, and therefore deal largely with more or less different bodies of men from those surveyed for hookworm. Thus, Camp Wadsworth had New York men earlier and Southern men later, reported in the hookworm survey. Similarly Camp Hancock had Pennsylvania men earlier, but the data on hookworm refer only to a survey at a later date of Southern men therein. In fact, in all camps the men surveyed for hookworm are mainly either Southern men or Northern men with at least six months' Southern service. In camps reporting large numbers surveyed for hookworm the data pertain to a large number of the same men included in Colonel Vaughan's table, and more of a common period than when smaller numbers are reported.

There is, however, no clear suggestion that the rate of morbidity and mortality is proportional to the rate of hookworm infection among the more heavily infected camps. Thus Camp Bowie, with 12.3 per cent. (13.5 per cent. for men with sick data) infection, has about the same record for pneumonia and for total morbidity and mortality as Camp Wheeler, in which, (on very limited data) the percentage of infection was 25.4 (patients) or 76.4 (troop survey). In the cases of Camp Travis and Camp Jackson the numbers surveyed for hookworm were adequate, and the methods comparable. The former had the lower rate of hookworm infection (10.1 per cent.), but the higher rate of hospital admissions, and incidence of pneumonia (78 as compared with 36). The mortality rate in pneumonia was approximately the same in both camps (10.6 and 10.7) ; the total mortality was, however, greater in Camp Jackson, but not in proportion to the higher hookworm infection.

The percentages of infection by hookworm, as compiled from available army data in troops from North Carolina and South Carolina, which contributed to Camp Jackson, S. C., were 31.8, 29.0 and 23.6 respectively, while those in Texas and Oklahoma contributory to Camp Bowie were 11.7 and 7. The intensity of infection was also heavier in the individual among men at Camp Jackson, as shown by

Fig. 1. Reproduced from Chart 3, Vaughan and Palmer (1918), with the addition of the percentages of hookworm infections drawn either from Camp surveys or routine examinations at Base Hospital, using in each case the most representative determination. These percentages are plotted, as in the other entries on the plot, proportionately on the basis of the highest record, which was 76.4 per cent. at Camp Wheeler. In the case of Camps Wadsworth and Hancock the hookworm surveys deal with an entirely different group of men, of southern origin in the main, while the data concerning pneumonia were gathered while northern troops predominated in the camps.

the finding of 53 worms per infected man, by Major Barber, in 22 men on autopsy at that camp, and of 155 worms per man, in the stools, among 69 whites on treatment. We have no autopsy data at Camp Travis, but in 70 men, whose stools were examined on treatment, the number of worms was less than 5 per man.

In Kelly Fields 1 and 2 the percentage of infection in 2,575 men was 10, and on the examination there of the stools of 267 men at treatment only 8 worms per man were recovered, and in 201 of the discharged only 5 worms or less. These light percentages and intensity were due to the preponderance of Texas and Oklahoma men and to the urban life, social status and other limiting factors among the Southern men from other states attached to the Air Service.

In the face of the lighter percentages and intensities in Camps Travis and Bowie, as compared with Camp Jackson, we find a heavier incidence of pneumonia, and as high or higher mortality therein, than in the more heavily infected men at Camp Jackson. Other factors than hookworm are doubtless operative to produce this relationship. Insofar as any significance attaches to these variations in percentages and intensity of hookworm infection it would appear that light infections may be as potent as heavy ones in inducing whatever sensitization to pneumonia exists, as a result of the parasite or its supposed toxins.

However, if we divide the more significant camps into two lists, the one including all camps reporting a higher rate of infection by hookworm than 10 per cent. and the other those with less than 10 per cent., we find the following groupings:

TABLE IX.

More than 10 per cent. infection by hookworm.

	Source of troops.	Per cent. hookworm.	Pneumonia (rate per 1,000).		Location of camp.
			Morbidity.	Mortality.	
Camp Bowie......	Ark., La., Miss.	12.3	96	20.0	Texas
Camp Wheeler....	Ala., Ga., Fla.	76.4	95	23.6	Georgia
Camp Travis.....	Okla., Texas	10.1	78	10.6	Texas
Camp Pike.......	Ala., La., Ark., Miss.	10.7	63	24.9	Arkansas
Camp Beauregard.	Ark., La., Miss.	10.5	42	15.0	Louisiana
Camp Sevier......	N. C., S. C., Tenn.	21.0	36	11.5	South Carolina
Camp Jackson....	Fla., N. C., S. C.	18.6	36	10.7	South Carolina

Less than 10 per cent. infection by hookworm.

Camp Cody......	Ia., Minn., Neb., S. D.	2.2	52	9.7	New Mexico
Camp Taylor.....	Ill., Ind., Ky.	2.9	37	5.4	Kentucky
Camp Lee........	Pa., Va., W. Va.	5.6	24	5.5	Virginia
Camp Logan.....	Ill.	2.4	16	1.0	Texas

The percentages of infection by hookworm in the second group apply in the main, in these camps, to men from the South, or to those who were in the hookworm area for more than six months. They do not in most cases represent the total camp population. A reference to Table VI will show more fully the nature of the data. The contrast between the two groups is striking. Heavy infection by hookworm is accompanied by heavy incidence, and heavy mortality by pneumonia. Camp Logan, located in the same general territory with Camps Travis, Bowie, and Beauregard, but filled with Northern troops in the main, had but 14, 21, and 38 per cent. respectively of the incidence and 5, 9, and 7 per cent. of the mortality by pneumonia, which these three camps, filled with Southern men from the hookworm area, exhibited.

A somewhat closer comparison of the incidence of hookworm and pneumonia in Northern and Southern men can be obtained, if the data are compiled by states instead of by camps. This is presented in Table X in which the incidence of pneumonia and measles is compiled, on the basis of nativity, and that of hookworm infection, on the basis of state of enlistment or residence. Although the bases of compilation are not strictly comparable, they are sufficiently so to be significant, since nativity and residence as to state, are coincident in the majority of cases. In the matter of the Northern men examined for hookworm, the data are mainly from men who had had six months or more of army service or residence in the South. The percentages of infection reported therefrom from Northern states arise from two sources, men of Southern birth or residence enlisting from the North, or Northern men with exposure in the Southern hookworm area. The percentages of infection reported from the Northern states, in consequence of these facts, are without doubt, abnormally increased, and should be interpreted only as indicating the *number* of men reported in those states, not as the *percentages,* for no adequate representation of Northern men, as a whole, is available. In an inspection of the data arranged by states (Table X), it is at once evident that the

TABLE X.

Lobar pneumonia, broncho-pneumonia, measles, and hookworm infection among U. S. troops according to nativity—Enlisted men.

State of birth.	Annual admission rate per 1000.			Hookworm.		
	Lobar pneumonia.	Broncho-pneumonia primary.	Measles compli-cated.	Per cent.	Positive.	No. men examined.
Alabama	.233	.029	1.35	29.4	656	2,223
Alaska	—	—	.08	0	0	1
Arizona	.049	—	.22	3.1	4	128
Arkansas	.128	.065	1.36	6.4	787	12,292
California	.045	.013	.34	2.1	11	527
Colorado	.043	.015	.26	2.4	9	368
Connecticut	.028	.006	.08	1.5	4	265
Delaware	.020	.015	.13	8.7	2	23
District of Columbia	—	—	—	1.6	2	121
Florida	.381	.042	1.45	31.8	1,202	3,778
Georgia	.153	.029	.88	32.6	1,265	3,872
Idaho	.043	—	.15	2.6	1	38
Illinois	.054	.008	.27	1.5	17	1,153
Indiana	.060	.017	.57	1.7	10	582
Iowa	.088	.010	.43	0.5	2	418
Kansas	.104	.013	.75	0.8	3	354
Kentucky	.111	.016	.82	16.3	376	2,301
Louisiana	.122	.056	1.14	27.3	2,010	7,348
Maine	.027	.008	.31	0.9	2	216
Maryland	.035	.009	.11	2.1	12	584
Massachusetts	.027	.004	.09	1.1	9	813
Michigan	.038	.007	.23	1.0	7	665
Minnesota	.043	.006	.21	1.5	5	325
Mississippi	.131	.038	1.26	27.1	2,358	8,684
Missouri	.101	.015	.61	1.8	41	2,326
Montana	.009	.001	.19	1.2	1	81
Nebraska	.128	.011	.49	0.9	3	345
Nevada	.049	—	.11	8.3	2	24
New Hampshire	.035	.012	.16	4.2	2	47
New Jersey	.022	.008	.06	0.3	1	284
New Mexico	.046	.018	.23	2.6	9	345
New York	.025	.006	.07	0.7	11	1,479
North Carolina	.056	.015	.79	27.2	3,402	12,558
North Dakota	.033	—	.22	0	0	82
Ohio	.042	.009	.18	3.5	45	1,277
Oklahoma	—	—	—	6.9	607	8,686
Oregon	.042	.009	.31	2.5	2	81
Pennsylvania	.030	.006	.08	0.4	4	1,074
Rhode Island	.018	.007	.04	1.4	1	73
South Carolina	.079	.018	.66	23.6	1,918	8,135
South Dakota	.053	.005	.32	1.6	1	63
Tennessee	.099	.027	1.21	12.3	1,233	9,722
Texas	.231	.054	1.58	11.7	3,494	29,837
Utah	.032	.005	.21	3.3	2	61
Vermont	.005	.002	.40	1.2	1	84
Virginia	.039	.011	.36	6.5	65	969
Washington	.059	.005	.11	2.2	3	137
West Virginia	.030	.009	.28	3.7	36	972
Wisconsin	.050	.009	.44	0.7	2	278
Wyoming	.041	—	.25	0	0	41
Totals	.067	.014	.43	3.3	19,732	594,238

states furnishing troops in which the infection was high, say above 20 per cent., are the very ones in which the two types of pneumonia and measles were most or at least highly prevalent. These states are seven—Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina.

The remaining states in the hookworm area, Arkansas, Kentucky, Missouri, (Oklahoma data lacking), Tennessee, Texas, Virginia, and West Virginia, carry a lighter infection and run a lighter incidence of the diseases named with some exceptions.

The states outside the hookworm area run a much lighter incidence of this parasite, which, when recorded therein, is probably in most cases introduced by migration and immigration. They also exhibit a noticeably lighter incidence of the pneumonias and of measles than those of the states of the hookworm area, excepting Virginia and West Virginia in which both hookworm and the diseases named are very light. This appears in the fact that while the minimum record for lobar pneumonia in the hookworm area, excluding Virginia and West Virginia is, .056 for North Carolina, it is surpassed outside that area in only five states, to wit, Indiana (.060), Iowa (.088), Kansas (.104), Nebraska (.128), and Washington (.059).

TABLE XI.

Summary of percentages of hookworm infections and incidence of pneumonia and measles.

	Lobar pneumonia.		Bronchopneumonia.		Measles.	
	Range.	Average per State.	Range.	Average per State.	Range.	Average per State.
Above 20 per cent. infection with hookworm, Ala., Fla., Ga., La., Miss., N. C., S. C.	.381–.056	.165	.056–.015	.032	1.45–0.66	1.07
Below 10 per cent., Ark., Ky., Mo., Tenn., Tex., Va., W. Va.	.231–.030	.105	.065–.009	.028	1.58–0.36	0.89
Total hookworm area	.381–.030	.134	.056–.009	.030	1.58–0.36	0.98
All other states	.128–.005	.043	.018–.001	.008	0.75–0.04	0.024

So also in the case of the incidence of broncho-pneumonia, the minimum rate in the hookworm area, as above limited, which is .015 in Missouri, is exceeded outside that area in only three states, to wit, Colorado (.015), Delaware (.015), and Indiana (.017).

Lastly, in the case of the incidence of measles in the hookworm area, as above limited, the minimum rate which occurs in Missouri (0.61) is exceeded outside that area only in Kansas (0.75).

There appears to be a tendency for certain states, mainly in the north central region west of the Mississippi River, to run a higher incidence of pneumonias and measles than states in the hookworm area, namely, those with only light hookworm percentages.

The above summary (Table XI) shows the range and average per state of the rates per 1000 admissions for lobar and broncho-pneumonia and for measles in the group of seven heavily infected states, in seven with lighter infections, in the hookworm area as a whole (fourteen states), and in the remaining Northern and Western states. Both the magnitude and direction of the differences, support in every instance the hypothesis that there is some correlation between the incidence of hookworm and that of pneumonia and

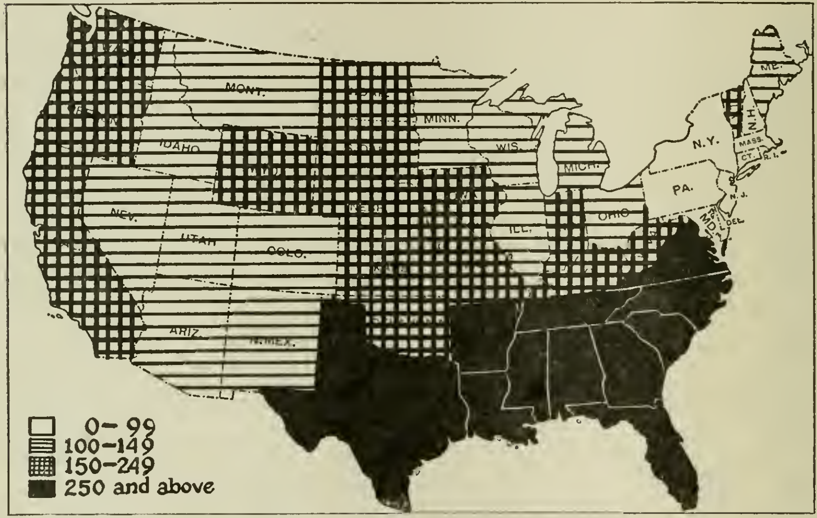


FIG. 2. Map of the United States showing the sum of admissions to sick report for primary lobar pneumonia; measles, simple or complicated; cerebrospinal meningitis and German measles. The chart gives for the different states the relative proportion of admissions to sick report which were for the named epidemic diseases. The table is drawn up by dividing the number of admissions in 1917 of the given diseases by nativity multiplied by 1,000 (Table 60, Surgeon General's Report, 1917), by the total admissions by nativity (Table 10, Surgeon General's Report, 1917). After Love and Davenport (1919, Fig. 8).

measles and suggest that the percentage of men infected, which appears to have a correlation with the intensities of their individual infections, is roughly proportional to the rate of admissions. In this connection we again revert to our recognition of the fact that hookworm infection is only one of a complex of factors, not the sole factor involved in this correlation. The data here presented in supplement to Colonel Vaughan's, strongly support the hypothesis of a correlation and offer a quantitative measure of it. In the first winter of the war, troops from the hookworm area had about three times the admissions (to army hospitals) for lobar pneumonia, and nearly four times those for broncho-pneumonia and for measles, than troops from outside that area.

TABLE XII.

Per cent. of urbanity in states of hookworm area, urban north, and rural western regions. Arranged from Davenport and Love (1919, Table 6).

Hookworm area.	Urban north.	Rural west.	Per cent. of urbanity.
Miss., Ark., N. C., S. C., Ala., W. Va., Okla.		S. D., N. D. (?), N. Mex., Nev.	10–19.9
Tenn., Ga., Va., Tex., Ky., Fla.		Ida., Neb., Kan., Wyo.	20–29.9
La.	Iowa	Ariz., Mont.	30–39.9
	Minn., Ind., Mo., Wisc., Mich., Vt., Del.	Ore., Utah	40–49.9
	Md., Me., Ohio, N. H.	Colo., Wash.	50–59.9
	Pa., Ill., Calif.		60–69.9
	N. J., N. Y.		70–79.9
	Conn.		80–89.9
	Mass., R. I., D. C.		90–100

In their exhaustive statistical study of immunity in urban recruits Love and Davenport (1919) conclude that life in urban communities produces a general resistance to disease of which the observed resistances to measles, mumps, lobar pneumonia, cerebro-spinal meningitis, and scarlet fever are only special instances. Their data and graphs exhibit in the main the heavier incidence of morbidity in the states of the hookworm area, as compared with that in the more urban northern states. It is, however, also significantly heavier in states in the hookworm area, than it is in the group of rural western states, whose degrees of urbanites are equivalent to or approach those of the states of the hookworm area, as shown in their figure 8 reproduced in our figure 2.

An examination of this figure and Table XII reveals clearly the fact that the sparsely populated rural states of the mountain region (except Wyoming) have a relatively low combined admission rate (below 149) for lobar pneumonia, measles, meningitis, and German measles, while the rural states of the hookworm area all have a high rate (in excess of 250), except the border states of West Virginia, Kentucky and Oklahoma, in which the percentage of infection by hookworm is relatively light. It appears from this contrast that hookworm may be the differential and potent factor contributing to this greater incidence in morbidity in the hookworm (rural) area, as compared with non-hookworm states, both rural and urban.

HOOKWORM AND INTELLIGENCE.

There is some quantitative evidence of the relation which these light infections, with which we are dealing to a considerable degree in Camp Bowie, bear to the mental status of recruits. An investigation of this relation was made by Major B. F. Pittenger and the senior author at Camp Travis, Texas, whose Depot Brigade received increments from the same territory from which the 36th Division at Camp Bowie was drawn. This deals with the comparative intellectual attainments of 9,254 men of the April, 1918, increments, including white and colored, literate (alpha test) and illiterate (beta test), those with hookworm and those reported negative for the infection.

This investigation has a bearing on the question of hookworm and morbidity, since it indicates intellectual inferiority of hookworm victims, as compared with apparent negatives. This inferiority is doubtless the result of a vicious circle of factors, of which hookworm is but one. As such it may operate by the action of the supposed toxins from the worm upon the central nervous system of the host. Action of the toxin upon the nervous system may also be a factor in this incidence of disease among hookworm subjects. The large size and relatively great volume of the glands opening into the buccal cavity of the hookworm, provide the basis for a relatively large secretion, whose absorption into the circulation brings this secretion in contact with the tissues of the central nervous system.

The following table (Table XIII) shows the number of men in the several classes enumerated above, the median of each group attained in the psychological test to which they were subjected, and the extent and percentage of mental deficiency, accompanying hookworm infection.

TABLE XIII.

Comparison of psychological records of 9,254 recruits at Camp Travis, Texas, with reference to hookworm infection, in white and colored troops, and literate and illiterate groups.

No. men.	Literacy.	Infection.	Race.	Alpha score. Weighted ratings. Median.	Beta score. Weighted ratings. Median.	Difference.		Stanford-Binet. Median.	Difference in score.
						Score.	Per cent.		
4,792	Literate	Negative	White	107.4	—	—	—	16.7	—
501	Literate	Positive	White	80.4	—	−27	−22.3	15.2	−1.5
823	Illiterate	Negative	White	—	47.1	—	—	13.0	—
131	Illiterate	Positive	White	—	40.9	− 6.2	−13.2	12.1	−0.9
1,817	Literate	Negative	Colored	28.6	—	—	—	11.6	—
74	Literate	Positive	Colored	27.5	—	− 1.1	− 3.9	11.5	−0.1
1,060	Illiterate	Negative	Colored	—	20.0	—	—	10.6	—
52	Illiterate	Positive	Colored	—	16.9	− 3.1	−15.5	10.9	−0.3

Both the test and the hookworm examination were given within approximately two weeks after arrival of recruits at Camp Travis, and prior to the treatment for hookworm. The results of the two types of examination are also expressed in terms of the common scale (Stanford-Binet) of the Manual of the Psychological Division, in years of mental development. It appears that the median alpha score of literate whites with hookworms, is 27 points or 22.3 per cent. below that of men in the same class in whom the infection was not detected, while in illiterates the difference in score is 6.2 or 13.2 per cent. In literate blacks, the difference is less, as might be expected, because of a relative racial immunity. The racial difference is more evident, if the results are reduced to the common scale (Stanford-Binet) in which the intellectual status is expressed in mental age. According to this, literate whites with hookworm are, in attainment, 1.5 years below those without it, and literate blacks only 0.1 year. Illiterate whites are only 0.9 of a year deficient and illiterate blacks 0.3, when infected with hookworm.

Compilation of the percentile distribution of scores, attained by positives and so-called negatives, shows that the mental deficiency in literate whites with hookworm, as measured by the falling off in score attained by ten groups of ten per cent. each, is proportionately somewhat the same for the uppermost ten per cent., as the successive groups below, there being a tendency, however, for the degree of the deficiency to increase as the scores fall. This is shown in the accompanying table and graph, in which the first entry shows the average score of the uppermost ten per cent. of the men, and following entries successive ten per cents. (Table XIV).

The interpretation of the data is that men infected with hookworm belong to all classes of mental rating, but not wholly proportionately in all groups to those without the infection, the men with low scores showing a greater relative deficiency as the score falls. It also appears that the infected men are pushed *en masse* below the normal by infection with hookworm, or by other factors correlated therewith in a vicious circle, but that the men with lower scores have a greater momentum, and move relatively farther from the norm, than do infected men with higher scores. This suggests the conclusion that

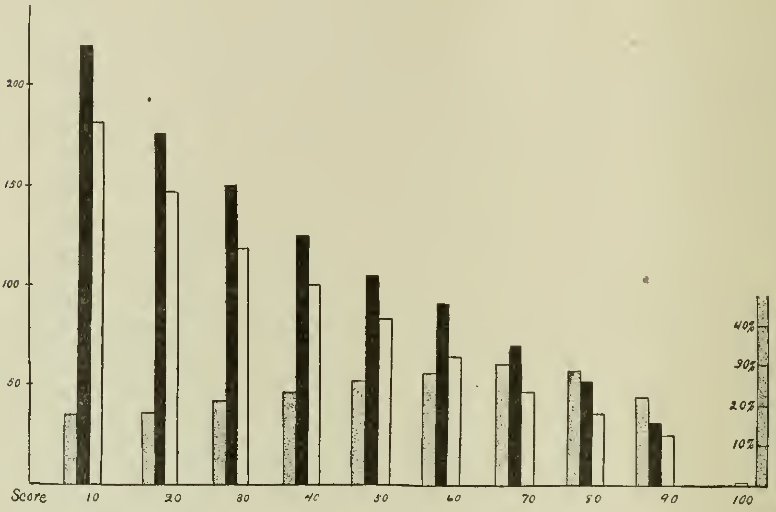


FIG. 3. Graphs showing percentage distribution of scores in psychological ratings of literate whites, of the April Increment in the 156th Depot Brigade, Camp Travis, Texas. The black column in the center of each group represents the median score of that percentage in men in whom hookworm had not been detected; the blank column at the right represents the median score for the corresponding percentage of men with hookworm, and the dotted column at the left represents the percentage which the deficiency in the score of the latter forms of the median score of the men without hookworm. The first group at the left thus represents the median score of the highest ten percentage, of the men without and of those with hookworm, and the deficiency of the latter in per cent. of the former. Succeeding groups represent successive ten percentages in similar fashion. The first ten percentages include the uppermost ten per cent. of each group, measured in terms of the psychological scores.

men with hookworm include a greater proportion of subnormal types than men without, and that subnormal men tend to acquire the disease more readily than men of higher categories. It is also possible that the intensity of individual infections may be heavier in the groups of lower intelligence, due to the relation of intelligence to sanitation, which in turn modifies the chance of infection and thus the ultimate intensity thereof.

TABLE XIV.

Percentile distribution of scores of literate whites, with and without hookworm, and the deficiency in score in points and per cent. of April increments, 165th Depot Brigade, Camp Travis, Texas, compiled by Major G. F. Pittenger.

Negatives.		Positives.		Mental deficiency.	
Per cent.	Score.	Per cent.	Score.	Points.	Per cent.
Highest 10%...	219	10	181	38.0	17.4
2d 10.....	178.5	20	146.6	31.9	17.8
3d 10.....	150.6	30	118.6	32.0	21.2
4th 10.....	127.7	40	99	28.7	22.5
5th 10.....	107.4	50	80.4	27.0	25.1
6th 10.....	89.6	60	63.5	26.1	29.1
7th 10.....	70.7	70	48.7	22.0	31.1
8th 10.....	51.3	80	36.1	15.2	29.6
9th 10.....	31.8	90	24.6	7.2	22.6
Last 10.....	0.0	100	1	0	0

From the time a man with hookworm infection enters the army, until his cure or discharge from service, he becomes a distinct liability. He tends to lower the morale and efficiency of his organization by having less capacity for continued, prolonged, or heavy work, by acquiring and reacting less favorably to prevalent diseases, by being off duty more frequently, by greater liability to severe or fatal illness, and by greater tendency to delinquency. He can be relieved of the parasites in a short time without being on sick call for more than two days for treatment. The time, labor and expense of repeated stool examinations to detect infections, and treatment, are small in comparison with the burden that hookworm seemingly creates, and the work can be systematized much the same as has typhoid and cowpox inoculation. Examination and treatment, if needed, for all men entering the army, at least from the hookworm area, should be a matter of routine and permanent record.

Should this be done for recruits early in their army career, the increased efficiency of the organization as a whole, and of the men individually, would fully justify the expenditure on grounds of cost, efficiency and morale.

Industrial enterprises in hookworm areas might raise the morale and increase the efficiency of their employes, and schools accelerate education by banishing hookworm.

SUMMARY.

1. This survey at Camp Bowie, Texas, utilized the results of an examination of one stool for hookworm ova from 22,842 men, with 3,079 or 13.5 per cent. reported positive, and 19,828 negative.

2. Among the men with hookworm, 1,767 were sick, with 4,993 appearances on sick call and 908 admissions to the hospital.

3. The so-called negatives had 9,091 sick with 17,494 sick calls and 3,421 admissions to the hospital.

4. The positives show an increase over the so-called negatives, of 27.9 per cent. in men sick, 88.6 per cent. in sick calls, and 76.5 per cent. in hospital admissions.

5. The same general direction of differences between men with and without hookworm appear in data obtained from the majority of individual companies making up the 141st Infantry and the 143d Infantry.

6. Positives for hookworm show an apparent increased susceptibility for tonsillitis, laryngitis, bronchitis, pneumonia, and measles. This is probably explainable as the result, in part, of rural isolation and resulting lack of immunities created by previous attacks, and by rural conditions favoring hookworm infection. The increased severity and chronicity of the respiratory infections in men with hookworm, as compared with men in whom the parasite was not found, may give rise to an apparent increase in susceptibility, or in "men sick," because light infections in men not having hookworm do not result in more than a temporary or a carrier phase of the infection, which wholly escapes medical attention and record.

7. The mortality in the ten heavily infected organizations, with hookworm above 10 per cent. is 1.5 per cent. In the seven more lightly infected organizations, with hookworm below 10 per cent., the death rate is 0.8 per cent., showing an increase in the more heavily infected group of 87.5 per cent. Morbidity and mortality from many causes, and the incidence of mortality from pneumonia, show a general correlation with the incidence of infection by hookworm, being higher as a rule wherever that infection is high.

8. The deaths from pneumonia in the 143d Infantry with 30 per cent. hookworm infection were 81, or 2.6 per cent., as compared with 187, or 0.8 per cent. in the remainder of the Division in which the infection by hookworm was 10.7 per cent. An increase of 284 per cent. in infection by hookworm in this regiment, as compared with the rest of the camp, is accompanied by a 325 per cent. increase in mortality by pneumonias.

9. Hookworm infections are relatively higher generally in hospital patients, among delinquents and in venereal isolation camps, than in camps, as a whole.

10. Troops from the hookworm area in army camps generally show a higher incidence of pneumonia and measles than do those from other regions. The incidence of these diseases is heavier in men from more heavily infected states, than in those from more lightly infected states.

11. Hookworm is one of a vicious circle of factors favoring inefficiency, morbidity, and mortality among army troops.

12. From the standpoint of economy and efficiency it would be more profitable to eliminate hookworm infection from every soldier, as he enters the service, than to allow him to continue untreated.

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RECENT EXPERIMENTAL STUDIES ON YELLOW FEVER.

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Plate V.

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The experiments of Reed, Carroll, Agramonte, and Lazear (1), carried out in Havana during the years 1900 and 1901, not only verified the theory of Carlos Finlay (2), that yellow fever is transmitted only through the bite of infected Stegomyia females, but they also established the exact conditions under which these infected mosquitos acquire and retain their infectivity for man. The value of these results, from the standpoint of sanitation, was of course very great. The rigorous antistegomyia campaigns initiated under Gorgas (3), first in Havana and then in Panama, led to the complete eradication of yellow fever from these two countries, notorious endemic centers of the disease since the time of Columbus.

Effective antistegomyia measures are capable of execution in those communities in which modern public health machinery is already in force or can be instituted, but in certain thinly populated regions with undeveloped sanitary organizations they are not always feasible. For example, in the tropical zone comprising Mexico and the Central and South American countries there still occur occasional epidemics of yellow fever which must have their origin in some small uncontrolled foci. The danger of an epidemic extending into any part of the United States through the introduction of an unnoticed yellow fever patient is remote, as its seaport cities and towns do not furnish the Stegomyia females, without which the disease cannot spread. The fact that certain endemic foci still exist in the southern republics keeps up our interest in yellow fever, and is a subject of grave concern for those countries in which it is present, since for new-comers it holds undiminished all the terrors that were so dreaded in past centuries. It impedes free communication between the interior and the coast of a country, retarding its development, and interferes with commerce among nations. Hence, notwithstanding the effectiveness of sanitary measures, it remains still one of the important infectious diseases, awaiting further extension of our means of combating it.

Beginning in 1918, the International Health Board of the Rockefeller Foundation initiated work against yellow fever in two directions: antistegomyia campaigns all over the world, and, in coöperation with the Rockefeller Institute, the investigation into the etiology of yellow fever. It was in connection with the latter activity that the writer was a member of three expeditions, the first to Guayaquil, Ecuador, in 1918, the second to Merida, Mexico, in 1919, and the last to Piura, Peru, in 1920. The present paper gives a brief review of the experimental studies made during these three expeditions.

From the work of Reed, Carroll, Agramonte, and Lazear, and also of later investigators, yellow fever was known to have the following characteristics: An attack confers a lasting immunity; the virus is present in the blood of the patient during the first 3 days of illness; the infection is carried from person to person through an intermediary host, *Stegomyia* (*Aëdes*) *calopus,* in whose body the virus undergoes change in such a manner, that after a period of about 12 days it becomes capable of passing into a non-immune person through the bite of the mosquito; the fomites and excreta are not infectious; the virus is easily killed by a temperature of 55° C. in 5 minutes; and it passes through the pores of bacteria-proof filters (Berkefeld). The virus had never been seen, however, and animal inoculations by various investigators had ended negatively, with the exception of those by Thomas (4), who succeeded in inducing, in monkeys and guinea pigs, a febrile reaction suggestive of yellow fever. Marchoux, Salimbeni, and Simond (5) observed that the virus in the blood, drawn from a patient, remains infective for several days, when kept under a layer of liquid paraffin at room temperature. Various bacteria described by Sternberg (6), Finlay (2), Sanarelli (7), and others were found to be secondary invaders. None of them produced the cardinal symptoms of yellow fever: typical temperature curve, hemorrhages, nephritis, and jaundice. Nor did any of them confer a lasting immunity, or belong to the filter passers. Sanarelli's *Bacillus icteroides* was identified later by Reed and Carroll (8) as a variety of hog cholera bacillus, and the *Bacillus X* of Sternberg as perhaps a member of the colon group. Seidelin's *Paraplasma flavigenum* (9) was shown to be not pathognomonic for yellow fever. Stimson reported in 1907 (14) the presence of a peculiar organism, which he designated (*?Spirochaeta*) *interrogans,* in the Levaditi sections of the kidney from a case of yellow fever occurring during the epidemic in New Orleans in 1905. He refrained from suggesting any etiological relation between this organism and yellow fever, as he was unable to extend his observations beyond this single case, owing to the lack of materials suitable for the application of the same staining method. No animal inoculations or culture experiments were undertaken by Stimson. In short, the causative agent of yellow fever was still unknown, when the American Commission* was sent down to Guayaquil in July, 1918.

The results of the Commission's investigations, including a clinical study of yellow fever by Dr. Elliott, and a bacteriological study by the writer, have already been in part published (10, 11).

Through the cooperation of Dr. León Becerra, Director of the Public Health Department of Ecuador, and Dr. Wenceslao Pareja, Director of the Yellow Fever Hospital of Guayaquil, the Commission was enabled to study a large number of yellow fever cases which occurred in Guayaquil during the months of July, August, September, and October, 1918. With few exceptions, these cases were all admitted to the Yellow Fever Hospital, where clinical studies by Dr. Elliott and Dr. Pareja, as well as bacteriological studies, were conducted. In the latter part of the investigation, due care was taken to select the early stages of the disease for study, while not excluding cases which had already passed the third day of illness. The main efforts were concentrated on the experimental reproduction of yellow fever in animals by inoculating them with the blood of yellow fever patients, or with cultures made from such blood. At the same time a search for an organism by means of the darkfield microscope was freely undertaken. It was the writer's view that neither animal inoculation experiments, especially through the intermediary of cultivation, nor darkfield search, had ever been exhaustively practiced by previous investigators. For this reason various birds, monkeys, cats, dogs, rabbits, and guinea pigs were employed.

In our early experiments one of the guinea pigs, brought from New York, developed typical symptoms and succumbed after having been inoculated with a culture made with the blood on the 5th day of illness from a fatal case. From this time on guinea pigs were principally used for the purpose of transmission, although other animals were occasionally employed in addition. When a suitable case was admitted blood was drawn from the median basilic vein, 2 to 5 c.c. of which was inoculated into each of two to four guinea pigs immediately, the remainder being used to set up cultures. A small portion of the blood, mixed with an equal part of a 2 per cent. sodium citrate solution was used for the darkfield examination. The medium used for cultivation was semisolid serum agar mixture, non-immune human serum being employed during the early period of the experiments, and fresh rabbit serum in the later work. The proportion of serum was 25 per cent., and that of the nutrient agar 0.2–0.3 per cent. After inoculation with the blood the media were covered with a layer of paraffin oil. At the beginning the cultures were kept at room temperature (about 20–25° C. in the day time) indifferently, but later in a thermostat, with a fairly constant temperature of 28° C.

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* The Yellow Fever Commission of the International Health Board was composed of Dr. Arthur I. Kendall, Dr. Charles A. Elliott, and Mr. Herman Edward Redenbaugh, of Northwestern University Medical School; Dr. Mario Lebredo, of Las Animas Hospital, Havana, Cuba; and Dr. Hideyo Noguchi, of The Rockefeller Institute for Medical Research, New York.

In all 27 cases were studied bacteriologically, with the following positive results.

Case 1 (fatal).—In a culture set up with the blood, drawn on the fifth day, a few motile organisms belonging to the Leptospira group were first observed under the darkfield after 5 days (July 24, 1918). Inoculation of the 3-days-old culture into 2 guinea pigs induced in both fever, hemorrhages, nephritis, and general jaundice. In the blood, as well as in the liver and kidneys leptospiras were demonstrated under the darkfield microscope. A pure culture of the strain was subsequently obtained.

Case 2 (recovered).—Blood drawn on the 4th day of illness (July 23, 1918) was inoculated into 2 guinea pigs, which showed a rise of temperature after 4 days. One was killed on the 6th day for transfer to two guinea pigs, one of which died with typical symptoms on the 7th day. The companion showed a transient fever, but died with an intercurrent infection. The leptospira was detected in the blood of the second transfer, and a culture obtained, which was later lost through contamination.

Case 3 (recovery).—Second day (Aug. 7, 1918) was inoculated into 3 guinea pigs, of which only one showed typical symptoms (9th day). The leptospira was found in the blood and organs of this animal and a culture obtained. A prolonged search of the blood of this patient, on the second day of illness, showed the presence of the leptospira.

Case 4 (fatal).—Third day blood (Sept. 4, 1918) was inoculated into 2 guinea pigs, one of which succumbed to typical infection in 12 days, while the other escaped infection. Leptospiras were found in the blood and organs of this patient. Transfers and cultures from the first guinea pig were successful.

This patient died on the 5th day, and the liver and kidneys were removed one hour *post mortem.* The emulsion of the liver contained a few leptospiras, that of the kidneys none. Inoculations of the liver emulsion were made into 4 guinea pigs, of which 2 died next day and one escaped infection. Only one guinea pig showed in due time typical symptoms, being distinctly icteric after 9 days, when killed for transfer. Leptospiras were found in the liver and kidneys, and a culture was obtained from a later passage.

Case 5 (fatal).—Sixth day blood (Oct. 3, 1918) was inoculated into 2 guinea pigs, one of which showed typical infection, while the other escaped infection. The first animal was intensely icteric when killed for transfer on the 8th day. The leptospiras were demonstrated and cultures subsequently obtained.

Case 6 (fatal).—Fourth day blood (Oct. 19, 1918) was used for cultures, no direct animal inoculation having been made in this case. Darkfield examination of the cultures after 7 days revealed a few motile leptospiras in one of the six tubes. Inoculations of the original culture and of subcultures of the strain finally secured its passage in guinea pigs, and its pathogenicity was established.

The findings just described established one concrete fact: that there existed in the blood of certain cases of yellow fever in Guayaquil a definite organism capable of inducing fever, hemorrhages, nephritis, and jaundice in guinea pigs. From its morphology and other characteristics the organism in question clearly belonged to the genus Leptospira, of which the causative agent of the infectious jaundice of temperate climates is also a member. From subsequent comparative immunological studies the leptospira from yellow fever cases is found to be closely allied to, but distinct from, that of infectious jaundice. The name of *Leptospira icteroides* was provisionally proposed for the organism.

The detection and isolation of *Leptospira icteroides* called for further investigations to determine the relation of the organism to yellow fever. As already mentioned, *Leptospira icteroides* produces in guinea pigs a rise of temperature to about 40° C., after a period of incubation, usually 3 to 5 days, rarely as long as 9 to 12 days. The fever lasts one or two days, and the temperature descends rather abruptly to normal and then to subnormal, with coincidental increase in nephritis, jaundice, and hemorrhage. Epistaxis is frequently observed in this animal. The nephritis and hepatitis are of acute parenchymatous nature, with various degenerative and necrotic alterations of the somatic cells. Jaundice is general. Hemorrhages are quite extensive, involving practically every visceral organ, and are notably severe in the lungs and gastrointestinal tract. The spleen is the least affected. Subcutaneous and intramuscular hemorrhages are constant. In human cases of yellow fever hemorrhages in various visceral organs are known to occur, especially in the gastrointestinal tract, in which they are almost constant, and less frequently and extensively in the lungs and subcutaneous tissues. This difference between the experimental leptospira infection in guinea pigs and yellow fever in man does not exist when marmosets or young pups are inoculated with *Leptospira icteroides*. In these animals hemorrhages are slight in extent. The lungs may show only a few small infarctions, and subcutaneous or intramuscular extravasation of the blood is slight (marmosets) or usually absent (pups). On the other hand, hemorrhages from the mucosa of the stomach and intestines are almost constant in these animals. Acute parenchymatous hepatitis and nephritis with jaundice and albuminuria are also constant in marmosets and pups. The pathological anatomical changes brought about by the leptospira infection in marmosets and pups resemble closely those found in human yellow fever.

As already noted, the virus of yellow fever was known to be a filter-passer, readily killed by a temperature of 55° C., capable of surviving in citrated blood, preserved under a layer of paraffin oil, for several days at room temperature, and transmitted through the infected *Stegomyia* (*Aëdes*) *calopus*. *Leptospira icteroides* has likewise been found to pass through bacteria proof filters, to be easily killed at 50° to 55° C. in 5 minutes, to survive for several days in citrated blood kept at room temperature, under a layer of paraffin oil, and finally, to infect *Stegomyia calopus,* when the latter are fed either on yellow fever patients during the early period of illness, or on guinea pigs experimentally infected. The mosquitos which have fed on blood from patients or infected animals acquire infectivity for susceptible guinea pigs, but it has not been determined what proportion of these mosquitos become infected. Transmission by means of the infected mosquitos was possible 8, 12, and 25 days after the time of feeding. In the body of these mosquitos leptospiras were found under the darkfield microscope. In the blood, as well as in the liver and kidneys of guinea pigs which developed typical symptoms and succumbed after being bitten by the mosquito leptospiras were also demonstrated. Whether or not *Leptospira icteroides* undergoes a life-cycle in the mosquito body, as is said to be the case with *Spironema recurrentis,* in the louse (Nicolle) (12) has not yet been determined.

If *Leptospira icteroides* is responsible for yellow fever in man, there should be a possible specific immunity reaction in the serums of yellow fever convalescents. For the purpose of deciding this point 18 convalescent serums were subjected to Pfeiffer's reaction with a strain of *Leptospira icteroides.* A positive reaction was obtained in 15 out of 18 cases, while 10 serums from persons who had never had yellow fever gave uniformly negative results.

Leptospira icteroides closely resembles *Leptospira icterohaemorrhagiae* of infectious jaundice, in its morphological and pathogenic properties, hence it was necessary to determine what immunological relations existed between these two varieties of leptospira. By means of monovalent immune serums, specific for each of the organisms, complement fixation, agglutination, and Pfeiffer reactions were carried out, with the result that anti-icteroides serum was found to have a powerful action upon *Leptospira icteroides,* but only a slight one upon *Leptospira icterohaemorrhagiae,* and vice versa. The Pfeiffer phenoenon, especially, showed the highest specificity. It was concluded, therefore, that these organisms are closely allied, but distinct species.

The brief exposition of the results of our investigation of yellow fever in Guayaquil shows that *Leptospira icteroides* had a definite etiological relation to the yellow fever then existing in that city. It was desirable, however, that similar studies be made of the disease elsewhere. The first opportunity for such study came when Mérida, the capital of Yucatan, Mexico, was visited by an epidemic of yellow fever in 1919–1920.

At the time of arrival of Dr. I. J. Kligler, of the staff of the Rockefeller Institute, who preceded the writer, the epidemic had almost subsided, but one new case and an autopsy were obtained for study. From the former, positive transmission to guinea pigs and subsequent isolation of *Leptospira icteroides* were successfully accomplished with blood drawn on the second and third days of the disease. A direct culture of *Leptospira icteroides* was also obtained from the patient's blood. Several of the guinea pigs, inoculated with the liver and kidney emulsions made from the organs of the patient autopsied (who had died probably on the 5th day of the disease), showed quite definite febrile reactions and characteristic lung lesions. In a few animals faint jaundice was noticed. Notwithstanding these symptoms and lesions no fatal leptospira infection could be induced by passages through guinea pigs, which either succumbed to secondary infection, or showed only a transient febrile reaction. No leptospira was observed with this material. The Pfeiffer phenomenon carried out with several convalescent serums collected in Mérida gave positive results with the Mérida strain, as well as with the Guayaquil cultures.

A second opportunity to extend our observations was available when, in 1920, an epidemic of yellow fever appeared in the northern part of Peru. The expedition (Dr. Kligler, and later also the writer) arrived there in time to study 14 typical cases of yellow fever. Because of adverse conditions and inadequate laboratory facilities (lack of suitable animals, means of sterilization, etc.) the results obtained in 9 cases studied at Payta were inconclusive. although there were several instances in which positive animal transmissions without fatal issue were brought about. The darkfield microscope was unavailable because of the lack of electric current, and staining facilities were not accessible, hence no search for the organism was made. Later, however, when the laboratory was removed to Piura, laboratory facilities were at hand. In the latter part of April, 1920, an epidemic was reported at Morropón, a town 65 miles from Piura, whither a party* went on horseback to obtain cultures. The transportation of laboratory materials and animals through the tropical desert was out of the question. Dr. Kligler utilized on this occasion fresh rabbit serum as culture medium to avoid the mishap which had occurred in Payta through the use of old rabbit serum. Blood cultures were made from five typical cases of yellow fever at Morropón, and carried back to the laboratory at Piura. Six to nine culture tubes were set up from each case.

On arrival at the laboratory the cultures were 8 to 11 days old, and had remained free from secondary contamination. Young, healthy guinea pigs, just brought from the United States were inoculated with the pooled contents of 2 to 3 tubes from each case. Positive transmission, resulting in fatal infection, after typical symptoms and with lesions, was obtained in all but one case, that is, in 4 cases out of 5. Darkfield examination revealed in the cultures from 3 of the 5 cases the presence of live leptospiras. From the successfully inoculated guinea pigs pure cultures of *Leptospira icteroides* were subsequently isolated. These strains were later identified as the same, as those derived from yellow fever cases in Guayaquil and Mérida. The Pfeiffer reaction was carried out with nine convalescent serums, five from individuals who had recovered from yellow fever a few months previously (epidemics of 1920 in Morropón and Payta), all of which reacted positively, and four from persons who had had yellow fever a year previously (1919 epidemic in Piura), of which only 2 gave positive reactions.

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* The party consisted of Dr. Enrico Caballero, the government expert stationed at Catacaos, Mr. John Mitchell, a sanitary engineer, and Dr. Kligler.

SUMMARY AND DISCUSSION.

Among the findings presented the following points are of considerable importance in a consideration of *Leptospira icteroides,* as the possible causative organism of yellow fever:

1. The presence of this organism in certain cases of yellow fever occurring in various localities (Guayaquil, Mérida, Morropón).

2. The similarity of the symptoms (period of incubation, character of fever, hemorrhages, particularly gastrointestinal, acute nephritis, acute hepatitis, non-participation of spleen, general jaundice, hypothermia towards death, brachycardia (13), etc.), between the experimental *icteroides* infection in animals and yellow fever in man.

3. The occurrence of the Pfeiffer phenomenon with *Leptospira icteroides* and the serum of individuals who have had an attack of yellow fever.

4. The transmissibility of yellow fever and the *icteroides* infection by *Stegomyia* (*Aëdes*) *calopus.*

5. The parallelism between the biological characteristics of the virus of yellow fever and *Leptospira icteroides* (filterability, thermal death point, invisibility under ordinary microscope, difficulty in the first transmission to animals, feeble resistance to putrefaction).

From the rather small percentage of fatal infections in guinea pigs following direct inoculation with the blood of patients, and also from the fact that a great many of those guinea pigs which do not succumb to the infection, nevertheless show, when killed, various lesions characteristic of leptospira infection, it may be deduced that guinea pigs are not always susceptible to the experimental transmission of yellow fever. It is probable that certain strains of *Leptospira icteroides* existing in the blood of yellow fever patients are not highly pathogenic for guinea pigs.

The leptospira is grown with difficulty in the first generation, whether from the infected animal or from the human being, and its rate of growth is much slower then than in later generations. It should be added also that favorable conditions of the culture medium are essential for the growth of this organism, particularly in the primary culture. Often among a large number of tubes only one or two show growth.

Since the foregoing article was written, Dr. Stimson has kindly given me the opportunity to examine his Levaditi preparations already referred to (14). The organisms are found most numerously along the free border of the renal epithelia of the tubules, and some in the interior of the cells; their distribution is irregular, and at some points several individuals are found in an entangled mass. The length of the organism is variable in different specimens but lies within a range of 6 to 14 microns. In the more heavily impregnated portions of the section the width of the organisms may be as great as 0.4 micron, in thinly impregnated specimens it is about 0.3 micron. They show minute elementary coils and irregular waves. The organism assumes the peculiar hooked formation of one or both ends, as described by Stimson. The general morphological features appear to be identical with those of *Leptospira icteroides*. In sections of material from experimentally infected animals (guinea pigs, puppies, marmosets) I have sometimes seen as many organisms as in Stimson's preparations, but I have not so far obtained as rich a preparation from human autopsy material.

It may also be noted here that during the Vera Cruz epidemic of yellow fever in 1920 Perez Grovas succeeded in transmitting the disease to guinea pigs, from which a culture of *Leptospira icteroides* was obtained (15). The writer received a Levaditi-Manouélian preparation from the liver of an infected guinea pig (courtesy of Dr. Perrin, of Mexico City) for comparison and is convinced that the morphological features are identical with those of other strains of *Leptospira icteroides.*

The accompanying microphotographs are appended for the purpose of comparative study of the morphological features of the strains of *Leptospira icteroides* obtained from different epidemics. Figs. 1, 2, and 3 stained by Levaditi's method, Fig. 4 by the Levaditi-Manouélian method (Dr. Perrin). Magnification × 1,000.

Fig. 1. The organism in the human kidney obtained by Dr. Stimson from a case of yellow fever occurring in the New Orleans epidemic of 1905.

Fig. 2. The organism in the liver of a marmoset infected with the Guayaquil strain of *Leptospira icteroides.*

Fig. 3. The organism in the liver of a guinea pig infected with the Guayaquil strain of *Leptospira icteroides.*

Fig. 4. The organism in the liver of a guinea pig infected with the blood of a yellow fever patient during the Vera Cruz epidemic of 1920.

Fig. 5. The organism in the kidney of a marmoset infected with the Guayaquil strain of *Leptospira icteroides.*

Fig. 6. The organism in the kidney of a guinea pig infected with the Guayaquil strain of *Leptospira icteroides.*

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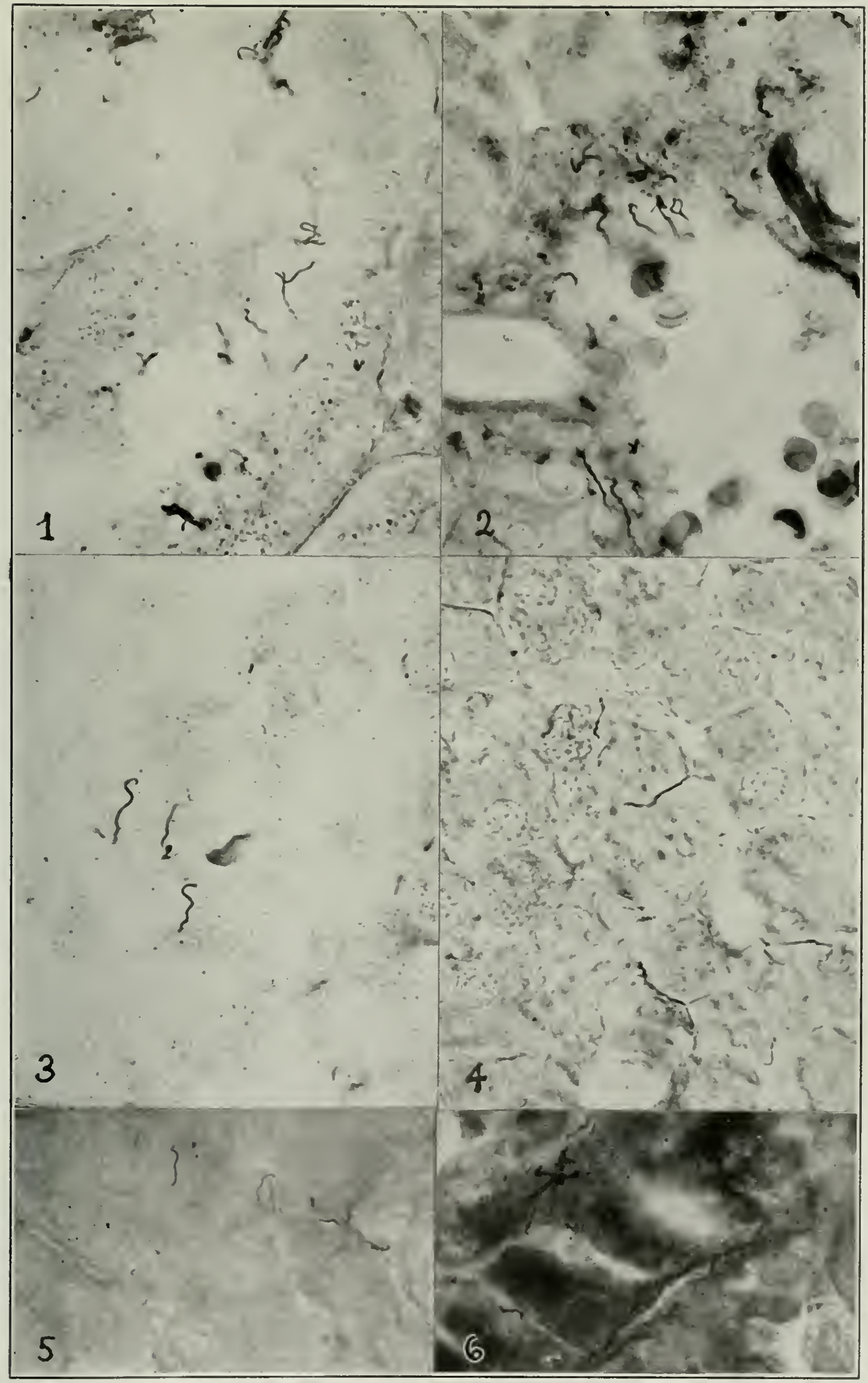

Plate V.

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EXPERIMENTS ON THE CARRIAGE OF INTESTINAL PROTOZOA OF MAN BY FLIES.*

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Plates VI to VIII.

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Introduction.

The danger to the public health arising from the pernicious activities of the common housefly and its relatives is. now well known. Numerous experiments, ably summarized by Graham-Smith (1914), have shown conclusively that flies may be important factors in the spread of such diseases as typhoid fever, paratyphoid, cholera and infantile diarrhea, in which the bacteria which cause the disease are found in large numbers in the feces of the person affected. These experiments have also shown, in great detail, how long these bacteria remain infective when carried by flies.

Besides these bacterial parasites of the human intestine, there are other parasites belonging to the group of the Protozoa. The world war gave a great impetus to the study of these comparatively neglected forms, and they are proving to be far more common in temperate climates than was formerly supposed. Amoebic dysentery, produced by *Entamoeba histolytica*, is the only disease which has been definitely proved to be due to an intestinal protozoan parasite, but it is highly probable that *Giardia* (*Lamblia*) *intestinalis* and perhaps other flagellates will be found capable of producing diarrheal symptoms under certain conditions.

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In the case of the intestinal bacteria carried by flies, it is known that the bacteria are unable to persist indefinitely within the intestine of the fly. Sooner or later they are killed, either by the action of the digestive secretions or by crowding due to the multiplication of the harmless bacteria which normally inhabit the fly's intestine. Although, as will be seen in the next section, considerable work has already been done on the carriage of the cysts of intestinal protozoa by flies, it has not yet been clearly shown whether the cysts pass through the fly and are deposited in its feces entirely unaltered, or whether they, like the bacteria, are ultimately killed by too long a sojourn within the alimentary tract of the fly. The solution of this problem is the main purpose of the present paper.

Historical.

The first attempts to test by experiment the possibility of the distribution of cysts of intestinal protozoa by flies occurred in 1913. Stiles and Keister (1913) proved that houseflies could carry "Lamblia spores" (the cysts of *Giardia intestinalis*) on their outer surface, and pointed out that, from epidemiological considerations, the carriage of cysts by flies from open privies to kitchens was almost undoubtedly the main avenue of infection in the part of the United States in which they were working.

In the same year, Kuenen and Swellengrebel (1913), in Java, carried on the first experiments on the carriage of cysts of intestinal amoebae by flies. They were also the first to use on cysts of intestinal protozoa the eosin test for viability (see section on methods), which has been used by all subsequent investigators. Their experiments with flies were not extensive. In one experiment, flies were much soiled with human feces containing cysts of *E. histolytica* and then examined for cysts. Three flies examined at once carried on their surface 25 living and 2 dead cysts, 41 living cysts, and 41 living and

3 dead cysts, respectively. Two more flies, examined one hour later, carried 27 and 52 cysts, all of which were dead. No cysts were found in the interior of any of the flies. In another experiment, flies allowed access for 48 hours to fecal material containing *E. histolytica* cysts showed no cysts, either externally or internally, when examined. Since they found no evidence of internal carriage, and had already found that cysts were killed in fifteen minutes or less by drying, these authors dismissed the carriage of cysts by flies as unimportant.

Thomson and Thomson (1916), in the course of an investigation into the intestinal protozoa of Egypt, noted that flies would greedily eat human feces containing protozoan cysts and that the cysts could be found in numbers in the alimentary tracts of the flies. They list the contamination of milk and drinking-water by flies among the methods by which cysts may be disseminated.

The most extensive piece of work on the carriage of cysts by flies is that of Wenyon and O'Connor (1917). Like Kuenen and Swellengrebel, they used the eosin test to determine whether cysts were living or dead. Their experimental results are summarized in Table I. The main points to be noted in these results may be listed as follows:

1. In most of their experiments, all the cysts found in the fly's intestine were recorded as living. In only two of their experiments was note made of the presence of dead cysts with the living ones.

2. The longest survival-time of cysts in the fly's intestine which they found was 24 hours for *Entamoeba histolytica* and *Giardia intestinalis*, 42 hours for *Entamoeba coli*.

3. Living cysts were deposited in the fly's feces for at least 16 hours after feeding, and motile forms of *Trichomonas* were found in the fly's feces 5 minutes after it had been fed on human feces containing these forms.

4. Cysts of *Entamoeba histolytica*, *Entamoeba coli* and *Giardia intestinalis* were found in the feces of flies caught wild.

In their own words, the conclusions from these experiments are given as follows (Wenyon and O'Connor, 1917, p. 145).

"(1) Flies feeding on feces readily take up encysted and other forms of protozoa into their intestine.

(2) The encysted forms of the protozoa can be found in the fly's intestine so long as any fecal matter remains there. If the flies are prevented from feeding this may be as long as forty-two hours.

(3) The cysts do not degenerate to any extent in the gut of the fly.

TABLE I.

Summary of the experimental results of Wenyon and O'Connor.

A. Flies fed on infected feces and dissected later.

Kind of cysts used.	Time between feeding and examination.	Number of flies whose intestines contained		
		Living cysts.	Living and dead cysts.	No cysts.
E. histolytica	18 hours	1	0	14
E. histolytica	20 hours	0	6	0
E. histolytica	24 hours	1	0	7
E. histolytica	42 hours	0	0	12
E. histolytica	70 hours	0	0	3
E. coli	24 hours	4	0	4
E. coli	42 hours	1	0	2
E. coli	70 hours	0	0	3
Giardia intestinalis	24 hours	0	5	3

B. Flies fed on infected feces and their own fecal droppings examined later.

Kind of protozoa used.	Time between feeding and examination.	Number of flies whose droppings		
		Contained living protozoa.	Were examined dry and dead cysts found.	Contained no cysts.
E. histolytica cysts	30 minutes	6	1	0
E. histolytica cysts	2 hours	4	0	0
E. histolytica cysts	5 hours	0	0	"small batch"
E. histolytica cysts	16 hours	6	0	0
E. coli cysts	40 minutes	6	0	0
Giardia intestinalis cysts	40 minutes	6	0	0
Giardia intestinalis cysts	5 hours	0	"small batch"	0
Trichomonas, motile	5 minutes	6	0	0

C. Examination of droppings of wild flies.

Total flies examined	229
E. histolytica cysts found in	5
E. coli cysts found in	6
Giardia intestinalis cysts in	1

(4) Flies can deposit in their own droppings material they have ingested only five minutes before."

From their conclusion (3) it is evident that Wenyon and O'Connor believe that protozoan cysts are not killed by a stay in the fly's intestine, as bacteria ultimately are. On the other hand, in two of their experiments both living and dead cysts were found in the fly's intestine. Wenyon and O'Connor used three species of flies, *Musca domestica*, *Calliphora* and *Lucilia*, the results being similar in all three.

Roubaud (1918) has also published a short paper on the rôle of flies as carriers of intestinal protozoa. This paper is in the nature of a summary and does not give the details of the actual experiments. He reports that:

1. Free amoebae (both *histolytica* and *coli*) fed to flies were found dead or moribund in the fly's intestine in less than an hour, never encysting.

2. Cysts of *Entamoeba coli*, *Entamoeba histolytica* and *Giardia intestinalis* traverse the digestive tract of the fly and are emitted, unaltered, with the fly's feces. They may be found in the feces for over 24 hours, but only rarely after 40 hours.

3. If a fly which has ingested cysts drowns in water, the cysts live for about a week, but are all dead by the time the fly decomposes enough to release them from its abdomen (this takes a month or more).

Roubaud used only *Musca domestica* in his experiments.

From the results of these various investigators, the only results so far available, it appears established that flies take up intestinal protozoa, both in the free and encysted state, from human feces; that the free forms die within an hour, but that the cysts persist in a living condition for 24 hours or more and are deposited, alive, in the fly's feces. These results also tend toward the view that the cysts are not harmed at all by their stay in the fly's intestine. This seemed remarkable, since the results in the case of intestinal bacteria are so different, and my own experiments were intended to test out this very point.

MATERIAL AND METHODS.

1. PROTOZOAN MATERIAL.—The protozoan material used has included free motile forms of *Entamoeba histolytica* and *Chilomastix mesnili*, and cysts of *Entamoeba histolytica*, *Entamoeba coli*, *Endolimax nana*, *Giardia intestinalis*, and *Chilomastix mesnili*. In experiments with the free forms and in a few of the cyst experiments, the flies were fed directly on a bit of fresh human feces containing the forms in question. In the majority of the cyst experiments, the cysts were concentrated and cleaned by a method devised by Dr. W. C. Boeck, now of the U. S. Public Health Service. This method involves emulsifying the stool in distilled water, straining the emulsion through several thicknesses of cheesecloth, throwing down the cysts and other solid particles by centrifugation and washing the sediment thus obtained (by repeated additions of distilled water, followed by cen-

trifuging or sedimentation and decantation of the supernatant liquid), until the water above the sediment appears clear to the naked eye. Material thus concentrated and washed will keep for several days or even weeks in a condition satisfactory for experimental work. Since this washed sediment has lost the odor which characterized the original fecal material, it was found advisable to add a bit of dilute syrup to this material before attempting to feed flies upon it.

I wish at this point to express my great indebtedness to Dr. W. C. Boeck for his kindness in furnishing me with cyst material and in discussing technique and results with me. My sincere thanks for assistance in securing material are also due to Dr. C. E. Simon and Dr. W. H. Taliaferro, of the School of Hygiene and Public Health, and to Dr. G. C. Payne of the International Health Board, now stationed in Trinidad, West Indies. Without the aid given by these gentlemen it would have been impossible for me to have carried out the series of experiments which I had planned.

2. Flies used.—The majority of my experiments were conducted with flies of the species *Musca domestica* (housefly) and *Calliphora erythrocephala* (blow fly) reared from larvae in the laboratory. A portion of the experiments were carried on with flies of other species, some caught wild and others reared in the laboratory. Other species used in small numbers were *Fannia canicularis* (lesser housefly), *Lucilia caesar* (green-bottle fly) and *Chrysomyia macellaria* (screw-worm fly). The results with all these species were similar. The larger green-bottles and blow flies ingested larger quantities of cyst material and retained it longer, and were therefore used for experiments involving a period of 40 hours or more between the time of feeding and time of examination.

3. Methods. (*a*) *Feeding flies.*—Flies were either used as soon as they had emerged from the puparium and become sufficiently hard to be handled, or were kept for several days in a large glass cylinder closed with netting and provided with a dish of syrup for food. In either case the flies to be used in an experiment were isolated in glass vials three quarters of an inch in diameter and two and a half inches long. When the flies had been transferred into these vials they were plugged with cotton and set aside for from 4 to 12 hours, so that the flies would be willing to feed readily. In feeding the flies, the cotton stopper was removed and the vial inverted over a glass slide on which had been placed a few drops of material containing the protozoa to be used. Ordinarily the fly discovered the food and

began to ingest it within a minute. Feeding continued for from one half to 3 minutes, depending on the size of the fly and the degree of starvation. When ingestion was finished the fly always left the food and walked up the side of the vial. At this point the cotton plug was again inserted and the vial set away until the fly was needed for dissection.

(*b*) *Examination of flies.*—Flies which had been fed on protozoan material were kept in the individual vials until examined. Where the time between feeding and examination exceeded 50 hours, the flies were fed once on sterile syrup about 30 to 36 hours after their feed on cysts. Even after this second feed, cysts could be found in the intestines of a small proportion of the flies. A fly which was to be examined was anaesthetized by putting a few drops of chloroform on the cotton plug of the vial. The fly was then removed from the vial, its legs and wings pulled off with forceps, and the body placed in a drop of normal salt solution on a slide. Under a binocular microscope the intestine was dissected out with spear-pointed needles and transferred to a drop of 1:10,000 eosin or neutral red solution on a second slide (Plate VI, Fig. 1). Still under the binocular, the intestine was carefully torn open and portions of the peritrophic membrane with the enclosed fecal masses were drawn out entire (Plate VI, Fig. 2). The remainder of the intestine was then removed and a cover glass pressed down on the drop containing the peritrophic membrane until the fecal masses were flattened out to about the thickness of a single cyst (Plate VI, Fig. 3). The fecal masses were then searched through with a compound microscope equipped with a mechanical stage. For this searching the 4 mm. objective and No. 10 ocular were used. The first fifty cysts encountered were taken as the sample and the number of these which remained unstained was recorded.

The use of a dilute solution of eosin as a test to determine whether a given protozoan cyst is living or dead was first recorded by Kuenen and Swellengrebel. It had been previously used by Strassburger and others in work on plant cells. Wenyon and O'Connor also used this test, concluding as a result of experiments designed to test its applicability that (Wenyon and O'Connor, 1917, p. 153):

> It seems, therefore, clear that the eosin-staining cysts are dead, though it may be argued that others which do not stain may be dead also or, at any rate, non-infective. Still, if we accept the eosin test as a criterion and regard all unstained cysts as living, the error in judgment will be on the safe side.

Very early in my own work it was found that a similar solution of neutral red stained dead cysts in the same way as the eosin solution, and that in comparative experiments a slightly larger proportion of cysts were stained by neutral red than by eosin. Since its greater transparency makes the neutral red solution easier to work with and since it appears to be a slightly more delicate test than the use of eosin, all the experiments reported in this paper were carried out with neutral red. In the earlier work comparative series were also examined in eosin, but since they showed only a consistent decrease of from 2 to 4 per cent. in the number of cysts stained, they were not included in the tables.

A cyst which has stained with neutral red can be easily distinguished from an unstained cyst. Not only can the red color be seen inside the cyst wall, but there is also a decided loss in refractivity, and, in many cysts, a decided plasmolysis and degeneration of the cyst contents. In cysts which have taken the stain only lightly, the most prominent differences are the lower refractivity and the appearance of the nuclei, which are usually readily visible in stained cysts but invisible or barely to be made out in unstained ones.

(*c*) *Numerical Expression of Results.*—In all previous work it has been assumed, seemingly, that the cysts all died at the same time, and as I have already brought out in my account of Wenyon and O'Connor's work, only twice did they record the finding of both stained and unstained cysts in their flies. My first series of experiments showed that this was not the case. During the time the cysts remain in the fly's intestine there is a gradual increase in the proportion of stained cysts until finally all are stained. In order to reduce this to a numerical expression I adopted the plan of counting the first fifty cysts seen in any preparation and recording the number of these cysts which remained unstained, which were presumably alive. Thus, throughout my tables, the numbers set below the various time intervals represent the number of living (unstained) cysts found in fifty cysts examined. Multiplying this number by two will in every case give the percentage of cysts still alive after this time interval. The number given under the heading "Original Material" represents in each case the number of unstained cysts found out of fifty cysts counted in a sample of the material fed to the flies, taken just before feeding began.

RESULTS.

1. *Survival of free forms of Entamoeba histolytica and Chilomastix mesnili in the fly's intestine.*

I have carried on only two experiments with free, motile forms. I include them here to confirm the similar observations of Roubaud on *Entamoeba histolytica* and of Wenyon and O'Connor on *Trichomonas.*

(*a*) *Entamoeba histolytica.*—Dec. 2, 1919. Five flies fed on drops of mucus taken from a dysenteric stool containing large numbers of motile amoebae with included red blood corpuscles.

Fly No. 1—examined after 7 minutes—amoebae unstained, some moving feebly.
Fly No. 2—examined after 20 minutes—none moving, most of them stained.
Fly No. 3—examined after 30 minutes—none motile, all stained.
Fly No. 4—examined after 1 hour—none motile, all stained.
Fly No. 5—examined after 1 hour—none motile, all stained.

This corroborates Roubaud's statement that free amoebae swallowed by flies die in less than an hour, never encysting.

(*b*) *Chilomastix mesnili.*—Nov. 8, 1919. Five flies fed on drops of a diarrheic stool containing large numbers of motile *Chilomastix.*

Fly No. 1—examined after 7 minutes—motile *Chilomastix* numerous, both in intestinal contents and in feces squeezed out of rectum.
Fly No. 2—examined after 20 minutes—a few specimens moving feebly, most of them non-motile and stained.
Fly No. 3—examined after 1 hour—all non-motile and stained.
Fly No. 4—examined after 1 hour—all non-motile and stained.
Fly No. 5—examined after 1 hour—all non-motile and stained.

This confirms Wenyon and O'Connor's findings with the closely related flagellate *Trichomonas,* showing that although the motile flagellates are killed in less than an hour, they may pass through the digestive tract and appear in the fly's feces in a few minutes.

2. *Survival of cysts of intestinal protozoa in the fly's intestine.*

(*a*) *Entamoeba histolytica.*—My experiments on the survival of the cysts of *Entamoeba histolytica* were all carried out on cysts from a single stool. Cases of amoebic dysentery are not common in Baltimore, and among the half dozen or so from which I was able to obtain specimens during the course of this work, only a single one proved to be a source of cysts. This stool was washed and concentrated on Dec. 9, 1919, and flies were fed on Dec. 9 (48 cysts out of 50 unstained), Dec. 10 (45 cysts out of 50 unstained) and Dec. 15–17 (37 cysts out of 50 unstained). The results of examinations for cysts are summarized in Table II. It will be observed that while no single series is complete, the different series supplement each other to such an extent that the composite record is reasonably complete, except for the gap between 24 and 42 hours. Although incomplete to this extent, the records show the same characteristics which appeared in all my experiments on the survival of cysts, and which may be summarized as follows:

TABLE II.

Survival of cysts of Entamoeba histolytica in the fly's intestine.

Original material.	Number of hours between feeding and examination.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
48......	48	48 48																		20
45......																	22	23		
37......							34	33		30	31	28	27 26		23 24				17	

Original material.	Number of hours between feeding and examination.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
48......	16			14 11																
45......		18																		
37......	14			13																

Original material.	Number of hours between feeding and examination.																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
48......																				
45......		6						2												
37......								2	1 1					0				0		

1. For a short time (2 hours in this case) no deaths occur, the number of cysts which stain being no greater than in the original material.

2. Next, for a time, there is a high death rate. The number of cysts which stain increases rapidly until (in 15 hours in this case) more than half of the cysts found are stained.

3. After this the cysts which still remain unstained die off more slowly, until finally all are found to be stained.

The results of the experiments on the survival of cysts of *En-*

tamoeba histolytica in the fly may be summarized in a single sentence: The experiments were carried out with cysts from a single source; cysts were found in 29 flies dissected at varying times after feeding; no deaths occurred for 2 hours; half the cysts were dead after 15 hours and the last living cysts found were in two flies examined 49 hours after they had fed on fecal material containing the cysts.

(*b*) *Entamoeba coli.*—Cyst carriers of *Entamoeba coli* are more numerous in Baltimore than carriers of *Entamoeba histolytica,* but for some reason, possibly because of the large size of the cysts, it was difficult to carry on experiments with the cysts of the former. Several times a large number of flies were fed on cyst material, but although their intestines contained abundant fecal material when examined, no cysts were found. It was finally discovered that when freshly emerged flies, either houseflies or blue-bottles, were given their first feed on cyst material, the cysts were ingested in large numbers. The results summarized in Table III were obtained in this

TABLE III.

Survival of cysts of Entamoeba coli in the fly's intestine.

Original material.	Number of hours between feeding and examination.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
46......		46	45	44		42	41									20				22
45......		45 44		40		42		40		30		30		25		20	22	19		18
40......	40	40 40 39		37 36 36 38		31										29 30 29 28 28		26 25		25 22 23

Original material.	Number of hours between feeding and examination.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
46......		19																		
45......		15		15 14		14 12		11 11		10 9		9		6						6
40......		22 20 20		19 19 17		15		11		9										

Original material.	Number of hours between feeding and examination.																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
46......								4							0					
45......		5		3		4														
40......		6		4		3 2 2		2		1		1 0					0			

way. The first successful experiments were carried out on Feb. 25, 1920 on material obtained in Baltimore (46 cysts out of 50 unstained). Then pressure of teaching duties delayed the resumption of experiments until June 7, 1920, when a second series was completed, also on Baltimore material (45 cysts out of 50 unstained). The remainder of this material was accidentally destroyed and the final series was carried out on July 6, 1920 on washed and concentrated material sent from Trinidad by Dr. G. C. Payne (40 cysts out of 50 unstained).

As can be readily seen from Table III, I was able to obtain much more complete series in the case of *Entamoeba coli* than in that of *Entamoeba histolytica.* Each of the three series is fairly complete, and in the composite series the largest gap is only six hours, from 34 to 40 hours after feeding. This series shows the same characteristics as in the case of *Entamoeba histolytica* and, as was to be expected from the close relationship between them and the similarity of their cysts, the results are almost identical with those obtained in working with the pathogenic species.

To summarize the work on *Entamoeba coli*, the experiments were carried out with cysts from three different sources; cysts were found in 75 flies dissected at varying time intervals after feeding; deaths were just beginning to occur at the end of two hours, half the cysts were dead in 14, 16 and 18 hours in the three different series; and the last living cyst was found in a fly of the third series, dissected 52 hours after feeding.

(*c*) *Endolimax nana.*—Cysts of *Endolimax nana* were not available at a time when I was able to carry on experiments with them until my work with the other species was completed. I can report only a single series of experiments with these cysts, the flies being fed on October 12 and 16 hours, the other between 32 and 37 hours. Since the cysts out of 50 unstained), furnished and identified by Dr. W. C. Boeck. The series is almost complete, there being only two gaps, one between 12 and 16 hours, the other between 32 and 37 hours. Since the cysts of *Endolimax nana* closely resemble those of the other intestinal amoebae, we would expect the time of survival in the fly's intestine to be about the same. The single series of experiments summarized in Table IV agrees closely with those carried out on the other amoebae, except that the last few cysts died off more rapidly than in the case of *Entamoeba histolytica* or *Entamoeba coli,* the last living cyst found occurring at 39 hours instead of somewhere around 50 hours. Since Dobell has found morphological characters which he considers justify the separation of *Endolimax nana* as the type of a new genus, *Endólimax,* it may be that this difference in survival time is significant. My scanty data do not, however, allow me to more than suggest this as a possibility.

TABLE IV.

Survival of cysts of Endolimax nana in the fly's intestine.

Original material.	Number of hours between feeding and examination.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
47......		47		45		41		36		34		31				27		25	25	21 18

Original material.	Number of hours between feeding and examination.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
47......	17	18		15		11		7		6		5					3		1	

Original material.	Number of hours between feeding and examination.																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
47......	0		0																	

To summarize, the experiments on the survival of the cysts of *Endolimax nana* were all carried out with cysts from a single source; cysts were found in 22 flies dissected; no deaths occurred for 2 hours, half the cysts were dead in 18 or 19 hours, and the last living cyst was found 39 hours after the fly was fed.

(*d*) *Giardia intestinalis.*—Cases in which infections with *Giardia* (*Lamblia*) *intestinalis* occur in children are not uncommon in Baltimore, and it was often possible to obtain good cyst material. These cysts are much less resistant to unfavorable conditions of any kind than those of the amoebae, and experiments had to be carried out as soon as the material came in, in order to have any considerable percentage of the cysts alive at the beginning of the experiments. The results summarized in Table V were obtained as follows: October 30, 1919, material from Baltimore (40 cysts out of 50 unstained); November 15–17, 1919, Baltimore material (40 cysts out of 50 unstained); November 18, 1919, same material as the last (31 cysts out of 50 unstained; November 21, 1919, same material as the last (27 cysts out of 50 unstained); November 5, 1920, material from Washington, D. C. (40 cysts out of 50 unstained).

TABLE V.

Survival of cysts of Giardia intestinalis in the fly's intestine.

Original material.	Number of hours between feeding and examination.																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
40....	40			38	34	29	29	18	14	11	8	15	12							0	0	
				39	33	31			20	16		6	2		2	1					0	
				35		37															0	
31....	31	30																	0	0	0	0
																			0	0		
27....		25							17	12	4	1										

As can be seen from the table, the cysts of *Giardia* succumb to the unfavorable influences in the intestine of the fly much more rapidly than do the cysts of the amoebae. Because of this high death rate throughout the stay of the cysts in the intestine of the fly, it is more difficult here to demonstrate that the last half of the cysts die more slowly than the first half. By making a rough interpolation, it can be said that 50 per cent. of the cysts die in seven and a half hours, while it takes eight and a half hours more before the remaining 50 per cent. are all dead, but the difference is not as evident as in the case of the other cysts.

Summarizing as before, cysts of *Giardia intestinalis* from three different sources were used in the experiments; cysts were found in 40 of the flies dissected; no deaths occurred for 1 hour, half the cysts were dead after 8 hours, and the last living cyst encountered was found in a fly dissected 16 hours after feeding.

(*e*) *Chilomastix mesnili.*—Through the kindness of Dr. C. E. Simon, Dr. Boeck and I were introduced to a gentleman with a chronic diarrhea accompanied by a severe infection with *Chilomastix.* Although we obtained stools from this case twice a week for several months, only on one occasion were cysts at all numerous. The series of experiments in Table VI were all carried out on this sample, the flies being fed on January 30 and February 1, 1920, at which time no stained cysts were found among the fifty examined. As the table shows, the cysts of *Chilomastix* are by far the most resistant of any with which I have carried on experiments, a few of them still being found unstained after 80 hours in the fly's intestine. The necessity of feeding the flies on sterile syrup during this period, in order to keep them alive, and the small percentage of flies in which cysts could be found after this feeding, made it impossible to obtain more frequent records in the latter part of the series. Here, even when starting with material in which no dead cysts were to be found, we see clearly that the additional time required to kill the last half of the cysts is greater than the time required to kill the first half. Half of the cysts, in this case, stained up at the end of 36 hours, but at the end of 44 hours more, two cysts out of fifty were still unstained.

TABLE VI.

Survival of cysts of Chilomastix mesnili in the fly's intestine.

Original material.	Number of hours between feeding and examination.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
50......		50		47		43		40		37		36		34		33		32		31

Original material.	Number of hours between feeding and examination.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
50......		29		28								26				25		24		

Original material.	Number of hours between feeding and examination.																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
50......								19						16						

Original material.	Number of hours between feeding and examination.																			
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
50......		12						10				7								2

Original material.	Number of hours between feeding and examination.									
	81	82	83	84	85	86	87	88	89	90
50..........						0				

To summarize: In the experiments with cysts of *Chilomastix*, the cysts came from a single source; cysts were found in 22 of the flies dissected; no cysts died for at least 2 hours, half of the cysts were dead after 36 hours, and the last living cysts found had been in the fly's gut for 80 hours.

3. *Survival of cysts in drowned flies.*

In order to test Roubaud's report that cysts of intestinal amoebae remained alive for a week or more in drowned flies, a single series of experiments were carried out with cysts of *Giardia intestinalis* and *Entamoeba histolytica*, which were fed to houseflies and bluebottles, and the flies then at once drowned in a vial of water. The results are given in Table VII. The results corroborate Roubaud's findings, some

TABLE VII.

Survival of cysts of Entamoeba histolytica and Giardia intestinalis in drowned flies

Species of cysts.	*Giardia intestinalis.*		*Entamoeba histolytica.*	
Species of fly.	*Calliphora erythrocephala.*	*Musca domestica.*	*Calliphora erythrocephala.*	*Musca domestica.*
Original material	30	30	45	45
Number of cysts remaining unstained after—				
1 day	26	26	40	38
2 days	24	20	33	34
3 days	12	16	26	24
4 days	6	8	19	20
5 days	0	0	11	13
6 days	0	0	5	4
7 days			2	1
8 days			0	0

of the amoeba cysts being still unstained after 7 days. I have already shown that the cysts of *Giardia* are less resistant than the amoeba cysts, so it is not surprising to find that the last living *Giardia* cysts were found on the 4th day. In this, as in all my other experiments, no difference was found between the behavior of cysts in different species of flies.

4. *Notes on feeding in flies, etc.*

I wish to treat briefly under this heading some points of interest that have come up during the course of this work, but which do not, strictly speaking, form a part of it.

(*a*) *How much food can a fly ingest at one time?*—Wenyon and O'Connor (1917, p. 143) attempted to determine how much fecal material a fly would take up during half an hour, by weighing small quantities of feces on a coverglass, exposing them for half an hour to some flies which had been starved for 2 or 3 hours, and then weighing them again, a deduction being made for evaporation on the basis of the loss in weight during the same time by a control preparation exposed under the same conditions except for the presence of the flies. Their results varied from 0.0005 to 0.0027 grammes per fly, averaging about 0.001 gm. per fly per half hour. This seemed to me a very small quantity, so I attempted to approach the problem in a somewhat different way.

A number of flies were starved over night (*i.e.*, from 17 to 21 hours) in individual vials. In the morning they were transferred into a single vial and weighed. Then they were transferred back into individual vials, fed individually on small separate drops of dilute syrup, transferred into the same single vial and weighed again. The weight of the syrup eaten was obtained by subtraction, and since the weight of a known volume of the syrup was easy to obtain, the volume as well as the weight of the food eaten could be calculated.

Two possible causes of error in this method are the soiling of the fly's surface with the food, and the deposition of fecal droplets in the individual vials before the flies were transferred into the vial in which they were weighed. As to the first, Graham-Smith (1910, p. 5) has already pointed out that flies fed on small separate drops of food-substances soil themselves very little, and I have also found this to be the case. Further, the ten to twenty minutes during which the flies which had been fed remained in their individual vials before they were transferred to the weighing vial, allowed a considerable time for cleaning. As to the second point, flies starved for as long a period as over night have usually emptied their intestines, and my experience has been that they do not ordinarily begin depositing fecal droplets for nearly an hour after they have fed. The results of these experiments were as follows:

House fly—*Musca domestica.*

1. 8 flies—starved 17 hours—gain in weight after feeding, 0.031 gm. or 0.004 gm. per fly; this corresponds to a volume of 0.0039 c.c.
2. 9 flies—starved 21 hours—gain in weight after feeding, 0.063 gm. or 0.007 gm. per fly; this corresponds to a volume of 0.0066 c.c.

Blue-bottle fly—*Calliphora erythrocephala.*

3. 5 flies—starved 17 hours—gain in weight after feeding, 0.117 gm. or 0.023 gm. per fly; this corresponds to a volume of 0.022 c.c.

Taken in conjunction with the results of Wenyon and O'Connor, these results point to the conclusion that the amount of food eaten by a fly at a single meal depends very largely on the length of time that it has been without food previously. Also, the larger amounts of food taken in by larger flies, like the bluebottles, must tend to increase their opportunities for carrying protozoan cysts and bacteria, and thus, to a certain extent, counteract their more infrequent occurrence in houses.

(*b*) *How soon after a fly has fed do cysts appear in the feces, and* *how long after feeding can they be found in the feces?*—All the work on dissection of flies which have been fed on cysts bears on this point, and I have also carried on some experiments in which flies were fed on dead yeast cells stained with neutral red. From these experiments I have concluded that the length of time which elapses between feeding and the deposition of feces depends very largely on the length of time since the flies have previously fed. A fly which has been starved for only an hour or so, may deposit feces immediately after feeding, and these feces may contain material that has just been eaten, as shown by Wenyon and O'Connor's observations on motile *Trichomonas*, and my own on motile *Chilomastix*. On the other hand, in a number of experiments where flies starved over night were fed on stained yeast cells, no fecal droplets were usually deposited for an hour after feeding, and the earliest fecal deposit noted in the whole series was 47 minutes after the conclusion of the feed.

In regard to the length of time during which cysts may be found in the feces, my experiments with stained yeast cells showed that if a fly is not fed again, the stained cells may be recognized in the feces up to the time of death, say about 48 hours, on the average. As I have already noted in the work on the survival of cysts, cysts can sometimes be found in the intestinal contents, and therefore undoubtedly in the feces, up to 86 hours after the infective feed, provided the fly is kept alive by feeding it on syrup in the meantime. Such cases are probably due to retention of some of the original feed in the crop of the fly, even after the intestines are practically empty. Graham-Smith (1914, p. 73) has noted this very phenomenon in flies fed on suspended carmine granules.

(*c*) *Notes on the occurrences of Herpetomonas in Baltimore flies.*—During my various experiments, I have dissected considerable numbers of flies and studied their intestinal contents carefully enough, so that a herpetomonad infection would almost assuredly have been noticed whenever it existed. It may be worth while to add the data on this point, for comparison with similar records from other parts of the world. My records include the dissection of 396 flies of the following species:

Calliphora erythrocephala	124
Musca domestica	225
Lucilia caesar	25
Chrysomyia macellaria	19
Fannia canicularis	3

TABLE VIII.

Survival of different protozoan cysts in the fly's intestine; averages compiled from Tables II to VI, inclusive.

Species.	Number of hours between feeding and examination.																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Giardia intestinalis	35.5	27.5		37.1	33.5	32.3	29	18	17	13	6	7.3	7		2	1			0	0	0	0						
Endolimax nana		47		45		41		36		34		31				27		25	25	19.5	17	18		15		11		7
Entamoeba histolytica	48	48					34	33		30	31	28	26.5		23.5		22	23	17	20	15	18		12.6				
Entamoeba coli	40	42.3	45	38.5		38.1	41	40		30		30		25		26.3	22	23.3		22		19.2		16.8		13.6		11
Chilomastix mesnili		50		47		43		40		37		36		34		33		32		31		29		28				

Species.	Number of hours between feeding and examination.																											
	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Giardia intestinalis																												
Endolimax nana		6		5					3		1		0		0													
Entamoeba histolytica														6						2	1					0		
Entamoeba coli		9.1		9		6						6		5.5		3.5		3.6		3		1		0.5			0	
Chilomastix mesnili				26				25		24										19						16		

Species.	Number of hours between feeding and examination.																							
	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Entamoeba histolytica		0																						
Entamoeba coli	0																							
Chilomastix mesnili						12						10				7								2

I have found only three cases of herpetomonad infection, one in *Musca domestica* (probably *H. muscae-domesticae*) and two in *Calliphora erythrocephala* (a larger species, probably *H. calliphorae*).

Discussion.

In order to be able to compare the times of survival of the different cysts within the fly's gut, I have averaged the results of the different series carried out on each species of cyst and summarized them in Table VIII. To permit of easier comparison, the graphs in Fig. 4 (Plate VII) were constructed from the data in this table. The numerous irregularities in the early part of the curves are largely due to the throwing together of different series, as can be seen, for example, by comparing the data on *Giardia intestinalis* in Table VIII with Table V, where the series are shown separately. The proportion of living cysts in the original material has a decided influence on the early portion of the curve, but appears to be less important in the last half of it.

As is plainly brought out in Fig. 4, the cysts studied fall into three distinct groups. The cyst of *Giardia intestinalis* is by far the least resistant, that of *Chilomastix mesnili* the most resistant. Intermediate between these two we find the amoeba cysts, *Entamoeba coli* and *histolytica* and *Endolimax nana*. The curves for all three species practically coincide up to about 25 hours, after which time the curve for *Endolimax nana* drops more steeply than those of *Entamoeba histolytica* and *coli*, ending at about 40 hours, as compared with about 50 hours for the other two species. Since my observations on the cysts of *Endolimax nana* were confined to a single series, this difference cannot be affirmed to have any significance. In view of the fact that the genus *Endolimax* has been separated from *Entamoeba* this apparent difference in the viability of the cysts is suggestive, but no more. Further work is needed to determine this point.

It is of great interest to note that Dr. W. C. Boeck, while working in this laboratory on the resistance of cysts to heat, secured results of the same general trend as my own. Dr. Boeck's work is soon to appear in this journal, but he permits me to state here that he finds that the cysts of *Giardia* die at a lower temperature than *histolytica* cysts, and these, again, at a lower temperature than the cysts of *Chilomastix*.

It seems probable, then, that the degree of resistance of cysts to various kinds of external influences is determined by some common factor, and it is at least a tempting hypothesis to suggest that this factor is the thickness of the cyst wall. While I have not attempted to measure the diameter of the cyst wall in any of these forms, a careful study of the living cysts certainly gives one the impression that the cyst walls of *Giardia* are thinner and the cyst walls of *Chilomastix* decidedly thicker than those of the amoeba cysts.

Turning to the practical aspects of the work, it is hoped that these results may be of interest to men working in the field on the general subject of soil pollution, first, because they emphasize again the importance of flies as carriers of disease-producing organisms from human feces to human food, and second, because they define more exactly the limits of such carriage, in so far as the cysts of intestinal protozoa are concerned. From this point of view, the relation of flies to the spread of intestinal protozoan infections may be stated as follows:

Flies feeding on a human stool containing cysts or free forms of intestinal protozoa will take large numbers of them into their intestines and deposit them again in their own feces. Since all stages of the protozoa are killed within a few minutes by drying, such fly feces are only dangerous to human beings when deposited on moist or liquid foods. A fly which has once ingested fecal material containing protozoa may deposit feces of its own which contain the infective forms of the protozoa at any time, from a few minutes after feeding until the most resistant forms, the cysts, are dead. The experiments reported in this paper show that the time of survival varies for the cysts of different species of protozoa and that the extreme limits are about as follows:

Free forms	1 hour or less.
Cysts of *Giardia*	16 hours.
Cysts of amoebae	40 to 50 hours.
Cysts of *Chilomastix*	80 hours.

These experiments also show that half of the cysts contained in the fly will be alive 7 hours after feeding in the case of *Giardia*, 14–16 hours after feeding for the amoebae and 36 hours after feeding for *Chilomastix*. The earlier hours, when a large proportion of the cysts are still living, must be far more dangerous than later hours.

Further, if a fly containing cysts is drowned in water, milk, soup or other liquid food, the cysts will live still longer, about a week, and there seems to be a possibility that human beings might be infected by swallowing such drowned flies.

SUMMARY.

1. Free forms of *Entamoeba histolytica* and *Chilomastix mesnili* ingested by flies were killed in an hour or less, not encysting. Motile *Chilomastix* were observed in a fly's feces 7 minutes after it had fed on a stool containing them.

2. Cysts of the intestinal protozoa ingested by flies were gradually killed in times varying with the species:

Entamoeba histolytica—half the cysts dead after 15 hours, last living cysts observed after 49 hours.
Entamoeba coli—half the cysts dead after 14 hours, last living cysts observed after 52 hours.
Endolimax nana—half the cysts dead after 18 hours, last living cysts observed after 39 hours.
Giardia intestinalis—half the cysts dead after 8 hours, last living cysts observed after 16 hours.
Chilomastix mesnili—half the cysts dead after 36 hours, last living cysts observed after 80 hours.

3. When flies which had ingested cysts were drowned in water, the cysts survived much longer:

Giardia intestinalis—half the cysts dead after 2 days, last living cyst observed after 4 days.
Entamoeba histolytica—half the cysts dead after 3 days, last living cyst observed after 7 days.

4. In experiments designed to test the maximum capacity of flies at a single meal, it was found that a single housefly (*Musca domestica*) could ingest 0.0068 c.c. of fluid and a single blow fly (*Calliphora erythrocephala*) could ingest 0.022 c.c.

5. An infection with *Herpetomonas muscae-domesticae* was found in only one out of 225 specimens of *Musca domestica* examined at Baltimore. Two infections with *Herpetomonas calliphorae* were found in 124 specimens of *Calliphora erythrocephala* examined.

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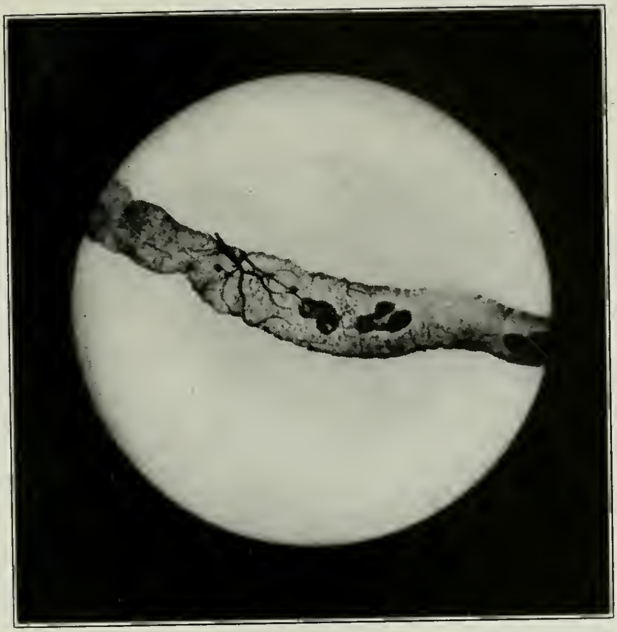

Fig. 1.

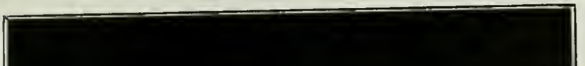

Fig. 2.

PLATE VI.

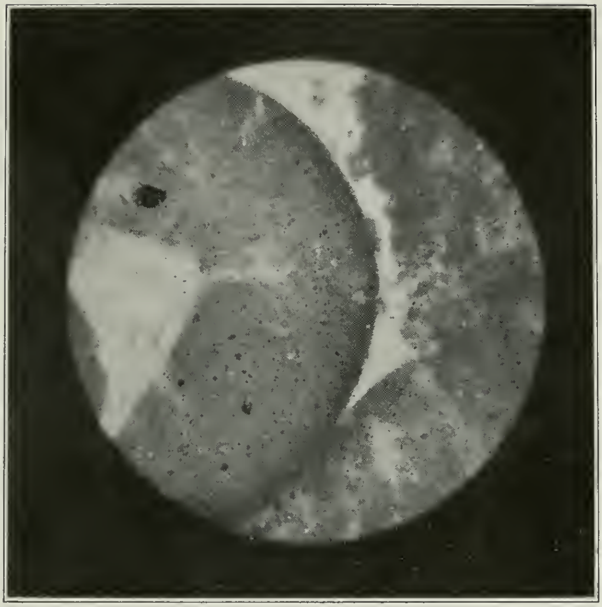

Fig. 3.

PLATE VII.

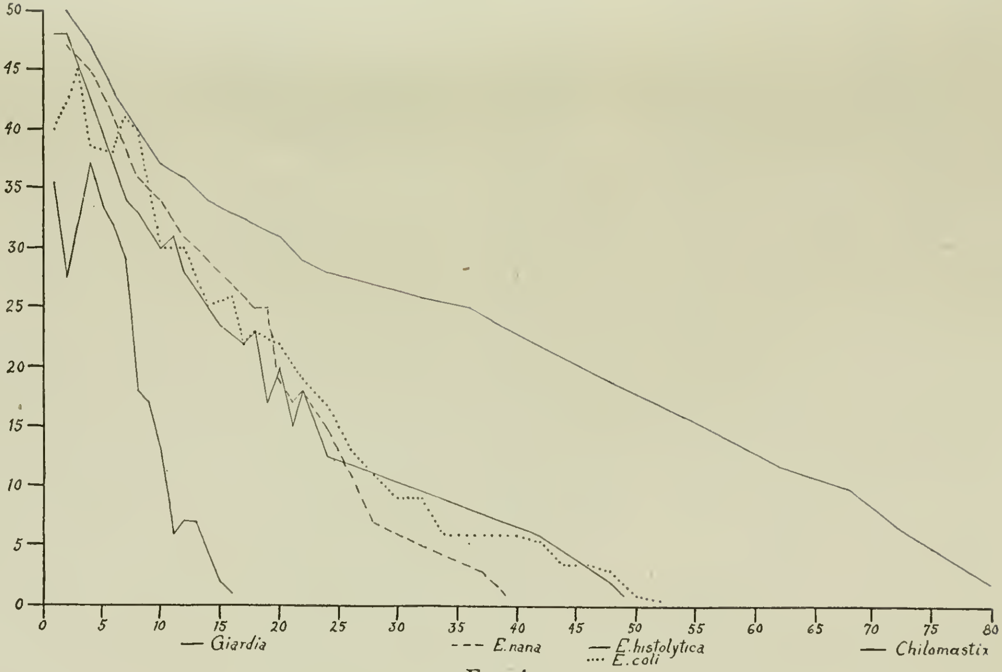

FIG. 4.

PLATE VIII.

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EXPLANATION OF PLATES.

FIG. 1. Photomicrograph of a small portion of a fly's intestine. The dark bodies showing through the intestinal wall are fecal masses.

FIG. 2. Photomicrograph of a loop of the peritrophic membrane, containing a number of fecal masses, which has been drawn out through a tear in the intestinal wall.

FIG. 3. Photomicrograph taken with much higher magnification than Figures 1 and 2, showing a portion of a single fecal mass, flattened out under a cover slip, but still enclosed within the peritrophic membrane. The scattered, oval, transparent bodies are cysts of *Giardia intestinalis* ingested fifteen hours previously.

FIG. 4. Graphs showing the gradual death of different protozoan cysts in the fly's intestine. Data taken from Table VIII. Ordinates show the number of cysts unstained out of fifty cysts examined, abscissae show number of hours between feeding and examination.

A COMPARATIVE STUDY OF HEMOLYTIC STREPTOCOCCI FROM MILK AND FROM HUMAN LESIONS.*

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The work presented in this paper was undertaken primarily to study the characters which differentiate hemolytic streptococci which are pathogenic for man from non-pathogenic hemolytic streptococci occurring in milk. Several papers in which the finding of hemolytic streptococci in milk is reported have appeared within the last few years. These streptococci are generally considered to be non-pathogenic, except in a few cases, in which hemolytic streptococci have been found in milk during epidemics of septic sore throat, and were thought to be the etiological agent in the disease.

Streptococci which produce a clear zone around the colony on a blood agar plate are usually regarded as hemolytic. There are wide variations among streptococci in their ability to produce zones of hemolysis. In fact, most streptococci and some pneumococci cause a slight clearing of blood agar around the colony. Smith and Brown (1) have studied the hemolytic zones made by streptococci on standardized blood agar plates, and have classified them according to the appearance of the zone of hemolysis. It was found that hemolytic streptococci of the typical human pathogenic type gave a perfectly well-defined zone of hemolysis, two to four millimeters in diameter. These streptococci were recognizable on blood agar plates, and were found to be a group with definite pathogenic properties. This group is designated by Smith and Brown as the Beta type.

It has been observed by several investigators, however, that streptococci which seem to have no pathogenic properties, but which produce, on blood agar plates, zones of hemolysis closely resembling the Beta type, frequently occur in milk. Davis (2) found streptococci of this kind in both raw and pasteurized milk, but more commonly in the former. Most of them were non-virulent for rabbits. Brown (3), in a recent paper, points out some of the characteristics which differentiate milk strains from human pathogenic strains.

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Preliminary examination of pasteurized milk in Baltimore showed that streptococci of the Beta type of hemolysis were present in the milk, in some cases in large numbers. A survey was made of the milk of a number of dairies to determine how generally these streptococci occurred. The bottled milk was bought from the dealers and plated on standard blood agar plates. Plain agar was used in the preparation of the blood agar. This was tubed in 12 c.c. portions, and to the melted and cooled agar 0.5 c.c. of whole rabbit's blood was added. Dilutions of the milk were made and plates were poured. The plates were incubated forty-eight hours at 37° C. The number of colonies showing hemolysis of the Beta type were counted and recorded. The numbers of these hemolytic streptococci in the milk of a given dairy varied widely from day to day. Also the proportion of hemolytic streptococci to other organisms varied widely. Table I represents typical counts of the milk of several dairies.

TABLE I.

The occurrence of hemolytic streptococci in milk.

Dairy.	Bacteria per c.c.	Hemolytic streptococci per c.c.	Per cent. of hemolytic streptococci.
A..........	45 000	3 600	8.0
B..........	22 000	320	1.4
C..........	157 000	340	0.2
D..........	28 000	None	0.0
*E..........	8 300	530	6.4
F..........	56 000	525	0.9
G..........	42 500	100	0.9
B..........	15 000	190	1.3
B..........	12 200	123	1.0
H..........	44 500	8 500	19.1
*E..........	6 950	225	3.2
D..........	26 000	None	0.0
B..........	22 500	100	0.4
I..........	55 600	None	0.0

The similarity of these pathogenic and non-pathogenic streptococci, on blood agar plates, and the frequency with which the latter occur in milk render the study of characteristics which differentiate them of importance.

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* Certified milk.

METHODS.

From a study of the literature on differentiation and classification of streptococci it becomes apparent that no single character will suffice to distinguish a group, though by the use of a group of characters it may be possible to define a fairly homogeneous group. In this work a study of the hemolytic streptococci which occur in milk was made with a threefold purpose in mind. It was purposed, first, to learn the characteristics of these streptococci and to see whether or not they constitute a homogeneous group; second, to determine the characters which are most useful in differentiating them from hemolytic streptococci which are pathogenic for man; and third, to study the thermal death rate of this group and of other hemolytic streptococci in milk. The following characters were selected for study:

1. Morphology.
2. Fermentation reactions.
3. Hemolysis.
4. Pathogenicity for mice.
5. Reduction of litmus and coagulation of milk.
6. Thermal death rate, in milk, at 60° C.

The milk strains used in this study were isolated from milk by plating on blood agar. Most of them were taken from bottled pasteurized milk, but some were from certified, unpasteurized milk. The blood agar was prepared by adding citrated whole rabbit blood to plain agar. One half c.c. of blood was added to each tube containing 12 c.c. of the melted and cooled medium. The dilutions of milk were made and then mixed with the agar on the plate. The plates were incubated 24 hours at 37° C.

Only those colonies which gave wide, clear, well-defined zones of hemolysis were selected. Colonies showing every degree of hemolysis from a barely perceptible halo to the wide, clear zones were found on most of the plates. Some colonies gave wide zones, but the outer limits of the zones were not well defined. Such colonies, when fished to broth and the broth tested with washed red cells gave no hemolysis. Only those which had the wide, clear-cut zones were found to respond to this test. These colonies correspond to Smith and Brown's Beta type. After a little experience it was found possible to pick these colonies with considerable accuracy.

The selected colonies were transferred to veal infusion broth. Smears were made from the broth cultures and examined for purity.

Stock blood agar slants were made from the pure broth culture. All tests were made upon transfers from these stock cultures.

For comparison with the milk strains a number of cultures from pathological sources were selected. Most of these were obtained from the Hygienic Laboratory of the United States Public Health Service, Washington, D. C. These cultures had been isolated mainly from empyema cases in army camps. Others were obtained from other sources, as shown in Table III.

TABLE II.

Milk cultures.

Culture.	Source.	Morphology.	
		Cells.	Predominating groups.
B 1...	Standard Pasteurized Milk	Oval	Short chains and pairs
B 2...	Standard Pasteurized Milk	Oval	Short chains and pairs
B 3...	Standard Pasteurized Milk	Oval	Long chains, cells paired
B 4...	Standard Pasteurized Milk	Oval	Short chains and pairs
B 5...	Standard Pasteurized Milk	Oval	Long chains, cells paired
F 4...	Standard Pasteurized Milk	Oval	Short chains and pairs
F 7...	Standard Pasteurized Milk	Oval	Short chains and pairs
F 8...	Standard Pasteurized Milk	Oval	Short chains and pairs
F 9...	Standard Pasteurized Milk	Oval	Short chains and pairs
F10...	Standard Pasteurized Milk	Oval	Short chains and pairs
F11...	Standard Pasteurized Milk	Oval	Pairs, a few short chains
F12...	Standard Pasteurized Milk	Oval	Pairs, a few short chains
F13...	Standard Pasteurized Milk	Oval	Pairs, a few short chains
F14...	Standard Pasteurized Milk	Oval	Pairs, a few short chains
C 4...	Standard Pasteurized Milk	Large oval	Pairs and short chains
C 7...	Standard Pasteurized Milk	Large oval	Pairs and short chains
C 8...	Standard Pasteurized Milk	Oval	Long chains
C 9...	Standard Pasteurized Milk	Oval	Short chains and pairs
C10...	Standard Pasteurized Milk	Oval	Short chains and pairs
W 1...	Certified Milk	Spherical	Long chains
W 2...	Certified Milk	Spherical	Long chains
W 3...	Certified Milk	Spherical	Long chains
W 4...	Certified Milk	Spherical	Short chains
W 5...	Certified Milk	Spherical	Short chains
W 6...	Certified Milk	Spherical	Short chains and pairs
W 8...	Certified Milk	Large oval	Long chains
W 9...	Certified Milk	Spherical	Short chains
D 6...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D 7...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D 8...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D 9...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D10...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D11...	Standard Pasteurized Milk	Spherical	Short chains and pairs
D12...	Standard Pasteurized Milk	Oval	Short chains and pairs
U 1...	Standard Pasteurized Milk	Oval	Short chains and pairs
U 2...	Standard Pasteurized Milk	Oval	Short chains and pairs
U 3...	Standard Pasteurized Milk	Oval	Long chains, cells paired
U 4...	Standard Pasteurized Milk	Oval	Short chains and pairs

MORPHOLOGY.

A study of the morphology of the milk strains was made soon after isolation. For this purpose they were grown in meat infusion broth, and stained by Gram's method, after 24 hours growth. The human strains were grown in broth and examined in the same way. Tables II and III show the summary of the points observed. Examination was also made of the growth from blood agar and from milk. In general it may be said that the organisms were more uniform in size and shape when grown in milk than when grown in other media. In broth, and especially on blood agar, the organisms tended to develop irregular shapes and sizes. This was more marked in the milk strains than in the human strains.

TABLE III.

Human pathogenic cultures.

Culture.	Source.	Morphology.	
		Cells.	Predominating groups.
24	Middle ear infection	Spherical	Long chains
177	Puerperal septicemia	Spherical	Long chains
179	Cerebrospinal meningitis	Spherical	Long chains
180	Scarlet fever	Spherical	Long chains
182	Septicemia	Oval	Long chains
235	Empyema	Spherical	Long chains
234	Empyema	Spherical	Long chains
238	Empyema	Spherical	Long chains, cells paired
240	Empyema	Spherical	Long chains
242	Empyema	Spherical	Long chains
250	Empyema	Spherical	Long chains
252	Empyema	Spherical	Long chains, cells paired
259	Empyema	Oval	Long chains, cells paired
260	Empyema	Spherical	Long chains, cells paired
261	Empyema	Spherical	Very long chains
200	Empyema	Large spherical	Long chains
247	Empyema	Spherical	Long chains
258	Empyema	Oval	Long chains and pairs
7	Septicemia	Spherical	Long chains
44	Septicemia	Spherical	Long chains
33	Empyema	Spherical	Long chains, cells paired
18	Septicemia	Spherical	Long chains and pairs
DB	Empyema	Spherical	Short chains
Viridans			
186	Pyorrhea	Spherical	Short chains
LI	Tonsilitis	Spherical	Short chains

General morphology and chain formation varied considerably among the various cultures of both groups. All cultures showed chain formation, but the proportion of the whole number of organisms in chains, and the number of elements in the chains were points of variation. In some of the milk strains pairs or diplococci predominated, while in others practically all of the organisms were in chains of several elements. The human strains were not so variable in this respect. Most of them were in chains of varying lengths. Variations in the size and shape of the cells were also more marked in the milk strains. Some showed small and delicately spherical cells, while others were larger and somewhat elongated.

The outstanding morphological differences between the organisms of the two groups may be summed up as follows:

Milk strains:

Cells elongated-oval, varying greatly in size; organisms less regularly in long chains, and whether in chains or not, the arrangement of cells was quite uniformly in pairs.

Human pathogenic strains:

Cells spherical, more regular in size and shape; chains usually long and few organisms not in chains; some, but not all, showed a tendency to diplococcus form.

Some cultures in each group are indistinguishable morphologically from those of the other group, but the majority of each showed the differences outlined. These characters remained constant, when observed in the same medium, after months of cultivation.

The morphology alone is thus of little value in distinguishing bovine from human strains but, when used with other characters, it will aid in the diagnosis of an unknown culture.

THE REACTION IN LITMUS MILK.

In the study of the reaction of streptococci in litmus milk, two distinct characters are involved, namely, the coagulation of the milk and the reduction of the litmus. Both have been found of some differential value by various observers. Observations which have been made on these characters are mentioned elsewhere. In addition it has been observed by several investigators, viz., Esten (4), Sherman (5), and Evans (6), that the behavior of streptococci of the lacticus type, in litmus milk, is characteristic. This characteristic reaction is used in some laboratories of dairy bacteriology to identify members of this group. The typical streptococcus lacticus, according to Evans, coagulates milk within 24 to 48 hours. This coagulation is accompanied by a reduction of the litmus, beginning at the bottom of the tube and progressing nearly to the surface, leaving a pure white column of milk with a pink layer on top.

All the cultures of human and milk strains of hemolytic streptococci were inoculated into litmus milk. The tubes were filled to a uniform depth, of about one and one-half inches, and the litmus in all tubes was as nearly as possible of equal concentration. The inoculated tubes were incubated at 37° C. and observed at frequent intervals. Record was made of the reaction after 24 hours and after 48 hours. The results are shown in Tables IV and V.

TABLE IV.

Reactions of pathogenic cultures in litmus milk.

Culture.	Reaction After. 24 Hours.	46 Hours.
24	No change	A
177	No change	A
179	No change	A
180	No change	No change
182	No change	No change
200	No change	A
233	No change	A
236	No change	A
240	No change	A
238	No change	No change
242	No change	A
247	A	A C
250	No change	No change
252	No change	No change
258	No change	A
259	No change	A
260	No change	A
261	No change	A
33	A	A
44	No change	No change
7	No change	No change
18	A	A C
106 (vir.)	R A C	R A C
DB	R A	R A C

R = Reduction of litmus.
A = Acid.
C = Coagulation.

TABLE V.

Reactions of milk cultures in litmus milk.

Culture.	Reaction After. 24 Hours.	48 Hours.
B 1	R A	R A C
B 2	R A	R A C
B 3	R A	R A C
B 4	R A	R A C
B 5	R A	R A C
F 6	R A	R A C
F 7	R A C	R A C
F 8	R A	R A C
F 9	R A	R A C
F 10	R A C	R A C
F 11	R A C	R A C
F 12	R A C	R A C
F 13	R A C	R A C
F 14	R A C	R A C
C 4	R A	R A C
C 7	R A	R A C
C 8	R A	R A C
C 9	R A	R A C
C 10	R A	R A C
W 1	No change	A
W 2	No change	A
W 3	No change	A
W 4	No change	A
W 5	No change	A
W 6	No change	A
W 8	No change	A
W 9	No change	A
D 6	R A C	R A C
D 7	R A C	R A C
D 8	R A C	R A C
D 9	R A C	R A C
D 10	R A C	R A C
D 11	R A C	R A C
D 12	R A C	R A C
U 1	R A C	R A C
U 2	R A C	R A C
U 3	R A C	R A C
U 4	R A C	R A C

R = Reduction of litmus.
A = Acid.
C = Coagulation.

It will be seen that the majority of the milk strains produce acid and cause a reduction of the litmus in 24 hours. Some cause coagulation of the milk also, but in most cases this occurred between the 24 and the 48 hour readings. Reduction of the litmus in the bottom of the tube appeared, in most cases, before any color change took place and by the time sufficient acid was formed to cause a pink coloration, only a layer about ⅛ to ¼ inch thick at the top remained colored, the rest being pure white. This shallow layer persisted for about three days and then deepened slowly, until at the end of a week about half of the column of milk was pink. The hemolytic streptococci from milk, therefore, give the same litmus milk reaction as do the members of the *S. lacticus* group.

Not all of the milk strains give this typical reaction, however. A small number acidify the milk very slowly, and cause no coagulation or reduction of litmus. Special mention should be made of the "W" strains. These are from a high grade of certified milk. This milk is produced under conditions which would, as nearly as possible by ordinary precautions, exclude organisms from such sources as the cow's flanks, the hand of the milker, the stable air, and unsterilized utensils. The organisms present would therefore be largely represented by those coming from the interior of the udder. Sherman and Albus (5) considered the streptococci obtained from the udder of the cow to be of the pyogenes type, and found them distinctly different, in a number of characters, from the lactic acid type. It is possible, therefore, that these milk strains which do not coagulate milk and reduce litmus are of the pyogenes type. The subsequent tests support this view.

The human pathogenic strains act slowly upon litmus milk. Little change is seen in 24 hours. Many of them show a weak acidity in 48 hours, while others cause little or no apparent change in the milk, even after several days incubation. Reduction of litmus was brought about by but one strain; two strains caused coagulation. Two strains of *S. viridans* which were included for comparison caused both reduction and coagulation.

In a general way, hemolytic streptococci from milk, produced under ordinary conditions, bring about a rapid acidification, coagulation, and reduction in litmus milk, while human pathogenic strains only produce a slight acidity. The exceptions, however, are too numerous to use this test for absolute differentiation.

FERMENTATION OF CARBOYHYDRATES.

The fermentation tests were made in a veal infusion broth which was prepared after the method described by Huntoon, that is, the broth was not filtered through cotton or filter paper, but was allowed to clarify by sedimentation in tall jars. This medium gave abundant growth of all strains without the addition of carbohydrates. In preliminary tests the broth was inoculated with *B. coli* to rid it of muscle sugar, but by comparison with controls this proved to be unnecessary and was abandoned. In all tests, however. control tubes to which no carbohydrate was added were inoculated and tested with the rest.

The test substances were made up in 10 per cent. solutions and sterilized separately. One per cent. of the test substance was added to the tubed and sterilized broth. The tubes were then incubated for 24 hours to insure sterility. Transfers were made to plain infusion broth from the stock cultures of the organisms to be tested. After 24 hours incubation 0.1 c.c. of this culture was added to each of the test broth tubes. All tubes were incubated for seven days at 37° C., including the tube from which seeding was made, this tube being the control mentioned above. Two drops of Andrade's indicator were added to each tube, at the end of the incubation period, and the tube was allowed to stand for a few minutes before being read. Little difficulty was encountered with doubtful reactions, nearly all being distinctly positive or negative. None of the controls showed any color with the indicator.

A tabular view of the results may be seen in Tables VI and VII. The only remarkable feature of these results is the uniformity with which saccharose is fermented by the human pathogenic strains, and the negative results obtained with this sugar when tested with the milk strains. The exceptions are nearly all in the "W" strains which resemble the human strains in their fermentation reactions. It was noted in the preceding test that they also reacted in litmus milk, as do the human pathogens.

These results are similar to those obtained by Sherman and Albus (5), who found that pyogenic streptococci fermented saccharose, while streptococci of the lacticus type did not. Their pyogenic cultures were isolated from the udder of cows, and were probably like the "W" strains in this table.

In the several classifications of streptococci, based on fermenta-

TABLE VI.

Fermentation of carbohydrates by milk cultures.

Culture.	Dextrose.	Lactose.	Saccharose.	Raffinose.	Salicin.	Inulin.	Mannite.	Control.
U 1.....	+	+	−	−	+	−	−	−
U 2.....	+	+	−	−	+	−	−	−
U 3.....	+	+	−	−	+	−	−	−
U 4.....	+	+	−	−	+	−	−	−
C 7.....	+	+	−	−	+	−	−	−
C 8.....	+	+	−	−	+	−	−	−
C 9.....	+	+	−	−	+	−	−	−
C10.....	+	+	−	−	+	−	−	−
F 7.....	+	+	−	−	+	−	−	−
F 8.....	+	+	−	−	+	−	−	−
F 9.....	+	+	−	−	+	−	−	−
F10.....	+	+	−	−	+	−	−	−
F11.....	+	+	+	−	+	−	−	−
F12.....	+	+	−	−	+	−	−	−
F13.....	+	+	+	−	+	−	−	−
F14.....	+	+	+	−	+	−	−	−
W 1.....	+	+	+	−	+	−	+	−
W 2.....	+	+	+	−	+	−	−	−
W 3.....	+	+	+	−	+	−	−	−
W 4	+	+	+	−	+	−	+	−
W 5.....	+	+	+	−	+	−	+	−
W 6.....	+	+	+	−	+	−	+	−
W 8 .	+	+	+	−	+	−	−	−
W 9 ...	+	+	+	−	+	−	+	−
D 7.....	+	+	−	−	+	−	−	−
D 8.....	+	+	−	−	+	−	−	−
D 9.....	+	+	−	−	+	−	−	−
D10.....	+	+	−	−	+	−	−	−
D11.....	+	+	−	−	+	−	−	−
D12.....	+	+	−	−	+	−	−	−

tion, this saccharose-negative, hemolytic streptococcus seems to have been overlooked. Andrews and Horder (7) have no saccharose negative groups, and Holman (8) does not use saccharose in his classification. In the latter classification, lactose and salicin-positive, mannite-negative, hemolytic streptococci, such as are shown in the table, would fall into the S. pyogenes group. So also would the human pathogenic strains and these two groups would not be differentiated.

It appears from the literature that the majority of milk streptococci ferment saccharose. *Streptococcus lacticus* is so described in textbooks. Savage (9) stated that most milk streptococci ferment saccharose. Houston (10) found this true of 90 per cent. of milk streptococci, while Broadhurst (11) found only 66 per cent. of milk strains saccharose fermenters.

Sherman and Albus (5), on the other hand, believe that typical *Streptococcus lacticus* does not ferment saccharose and their experiments seem to show that the fermentation of this sugar distinguishes *S. pyogenes* of cows' udders from *S. lacticus*.

TABLE VII.

Fermentation of carbohydrates by human pathogenic cultures.

Culture.	Dextrose.	Lactose.	Saccharose.	Raffinose.	Salicin.	Inulin.	Mannite.	Control.
24	+	+	+	−	+	−	−	−
177	+	+	+	−	+	−	−	−
179	+	+	+	−	+	−	−	−
180	+	+	+	−	+	−	+	−
182	+	−	+	−	+	−	+	−
200	+	+	+	−	+	−	−	−
233	+	+	+	−	+	−	−	−
234	+	+	+	−	+	−	−	−
238	+	+	+	−	+	−	−	−
240	+	+	+	−	+	−	−	−
247	+	+	+	−	+	−	−	−
242	+	+	+	−	+	−	−	−
250	+	+	+	−	+	−	+	−
252	+	+	+	−	+	−	−	−
258	+	+	+	−	+	−	−	−
259	+	+	+	−	+	−	−	−
260	+	+	+	−	+	−	−	−
261	+	+	+	−	+	−	−	−
44	+	+	+	−	+	−	+	−
33	+	+	+	−	+	−	−	−
7	+	+	+	−	+	−	−	−
DB	+	+	+	+	+	+	−	−
LI (vir.)	+	+	+	+	+	−	−	−
166(vir.)	+	+	+	+	+	+	−	−

From the data, presented in Tables VI and VII, it appears that saccharose may be useful in differentiating non-pathogenic hemolytic streptococci of milk from those of human origin.

HEMOLYSIS.

As stated before, all of the strains used in this study were isolated by picking typical hemolytic colonies from blood agar plates. In so doing only those colonies were selected which gave the Beta type of hemolysis (Smith and Brown). In the appearance on blood agar plates, therefore, all of the milk strains were practically identical, and they proved to be identical in appearance with the human pathogenic strains when grown on blood agar plates prepared in the same manner. The two groups were therefore indistinguishable by blood agar plate hemolysis.

All cultures were then further tested for the production of a soluble hemolysin in broth, as recommended by the medical department of the United States Army in a leaflet entitled, "The Isolation and Identification of Streptococcus Hemolyticus." The cultures were grown in infusion broth for 18 to 24 hours. One-half c.c. of broth culture was then added to one-half c.c. of a 5 per cent. saline suspension of washed rabbit red blood cells and incubated for two hours. Positive controls of known hemolytic cultures and negative controls with sterile broth were included in each test. Only those cultures which gave complete hemolysis in two hours were retained for study. All of the colonies which gave a wide, absorbing clear zone with well-defined limits responded to this test. Occasionally colonies were picked which gave wide, clear zones of hemolysis, but which, on close inspection showed some red color remaining in the zone. These cultures always gave negative results in broth. Others again had wide, clear zones but the outer border was not sharply defined. These also gave negative results in broth.

After some study, one may predict with considerable accuracy the reaction in broth by the appearance of the zone of hemolysis on a standard blood agar plate. The test for hemolysin in broth culture, therefore, seems to correlate with a definite appearance on blood agar plates and separates a group of hemolytic streptococci with definite hemolytic properties. It gives clear-cut results, that is, distinctly positive or negative.

It seems preferable to use this test to confirm results obtained with the blood agar plate. Variations in technique and in the personal judgment of different investigators render the reports of types of hemolysis on blood agar plates too uncertain. If a culture is reported as positive or negative in the broth test, its hemolytic properties are more clearly defined. Swabs were taken from ten normal throats of a class in bacteriology. All showed hemolytic colonies in varying numbers. The zones of hemolysis were wide, with well-defined borders. They were hardly distinguishable from the true Beta type. None of the ten, however, gave positive results in broth cultures. This suggests that perhaps the widely varying results reported in regard to the occurrence of hemolytic streptococcus in normal throats may be due to variations in what are considered to be true hemolytic streptococci.

It is evident that neither of the hemolysis tests mentioned suffices to distinguish Beta hemolytic streptococci from milk and from human lesions. Brown, in a recent paper, noted that cultures of milk strains

TABLE VIII.

Rate of hemolysis with human and bovine strains.

(Hemolysis after thirty minutes.)

Milk culture.	Dilution.					Human culture.	Dilution.				
	1/2	1/4	1/8	1/16	1/32		1/2	1/4	1/8	1/16	1/32
U 1...	++	+				24...	++++	+++	+++	++	+
U 2...	++	+				177...	++++	++++	+++	++	+
U 3...	+					179...	++++	++++	+++	+++	
W 1...	++++	++++	+++			180...	++++	++++	++++	++++	+++
F 7...	++	+				182...	++++	++++	++++	++++	+++
F 8...	+					233...	++++	+++	+++	++	
F 9...	+	+				234...	++++	+++	+++	+	
F10...	++					238...	+++	+++	+++	++	+
C 8...	++					240...	++++	++++	+++	++	+
C10...	++					242...	++++	++++	+++	++	
D 6...	+					33...	++++	++++	++++	++++	+++
D 7...	++	+				259...	++++	++++	++++	++++	+++
D 8...	++++	+++	+			260...	++++	++++	++++	++++	+++
D12...	++	+				261...	++++	++++	++++	+++	++
W 2...	+++	++				252...	++++	++++	++++	++++	++
W 3...	++	+				258...	++++	++++	++++	+++	++
B 3...	+++	++	+			247...	++++	++++	++++	+++	++
B 4...	++	+				250...	++++	++++	++++	++++	+++
B 5...	++	+				DB...	++	++	+		
B 1...	+					7...	++	++	+		
D10...	+					44...	++++	++++	++++	++	+
D 9...	+										
W 8...	++++	++									
W 6...	++++	++++	++++	++++	++++						
W 3...	++++	++++	++++	++++	+++						
D11...	+++	++	+								
F12...	++	+									
F11...	+	+									

TABLE IX.

Rate of hemolysis with human and bovine strains.

(Hemolysis after ninety minutes.)

Milk culture.	Dilution.					Human culture.	Dilution.				
	1/2	1/4	1/8	1/16	1/32		1/2	1/4	1/8	1/16	1/32
U 1...	++++	++++	+++	++	+	24...	++++	++++	++++	++	+
U 2...	++++	++++	++++	+++	++	177...	++++	++++	++++	++++	++
U 3...	++++	++++	++++	+++		179...	++++	++++	++++	++++	+++
W 1...	++++	++++	++++	++++	++++	180...	++++	++++	++++	+++	++
F 7...	++++	++++	++++	++++	++	182...	++++	++++	++++	++++	++++
F 8...	++++	++++	++++	++		233...	++++	++++	++++	+++	++
F 9...	++++	++++	++++	++		234...	++++	++++	++++	+++	++
F10...	++++	++++	++++	++		238...	++++	++++	++++	++	
C 8...	++++	++++	++++	++		240...	++++	++++	++++	++	++
C10...	++++	++++	++++	++		242...	++++	++++	++++	++	+
D 6...	++++	++++	++++	++++	++++	33...	++++	++++	++++	++++	++++
D 7...	++++	++++	++++	++++	++++	259...	++++	++++	++++	++++	+++
D 8...	++++	++++	++++	++++	++++	260...	++++	++++	++++	++++	++++
D12...	++++	++++	++++	++++	++++	261...	++++	++++	++++	++++	+++
W 2...	++++	++++	++			252...	++++	++++	++++	++++	++++
W 3...	++	++				258...	++++	++++	++++	++++	++
B 3...	++++	++++	++++	++++	++++	247...	++++	++++	++++	++++	++
B 4...	++++	++++	++++	++++	++++	250...	++++	++++	++++	++++	++
B 5...	++++	++++	++++	++++	++++	DB...	++++	++++	++		
B 1...	++++	++++	++++	++++	++++	7...	++++	+++	+		
D10...	++++	++++	++++	++++	++++	44...	++++	++++	++++	++++	++
D 9...	++++	++++	++++	++++	++						
W 8...	++++	++++	++++	++	+						
W 6...	++++	++++	++++	++++	++++						
W 5...	++++	++++	++++	++++	+++						
D11...	++++	++++	++++	++++	++++						
F12...	++++	++++	++++	++++	+++						
F11...	++++	++++	++++	++++	+++						

of the Beta type require a longer period of incubation with the red cell suspension to produce hemolysis. This fact had often been observed in the present work, and an experiment was planned to compare the two groups of cultures in this respect, and also to compare their hemolytic titer. Tables VIII and IX show the results of the test. The cultures were diluted with saline, incubated at the same time in a 37° C. water bath, and the results read at the end of 30 and 90 minutes. It will be seen that the milk strains have a very low titer after 30 minutes, while with the human strains hemolysis is nearly complete. The one "W" strain included resembles the human strains as in previous tests. After 90 minutes there is no striking difference in the hemolytic titer of the two groups. These results agree with those of Brown (3), and it is evident that the rate of lysis of blood cells is more rapid with human strains.

Another observation in connection with tube hemolysis of the milk strains should be noted, namely, the production of brownish discoloration of the cells before they are laked. This occurred with all of the milk strains, although it was more pronounced in some than in others. The color remained and became intensified after the lysis of the cells. Occasionally some of the human strains showed a slight browning of the hemoglobin. Spectroscopic examination of the brown colored tubes showed the characteristic absorption bands of methemoglobin in the red, yellow, and green.

PATHOGENICITY FOR MICE.

Streptococci vary so greatly in their pathogenicity for animals that the value of inoculation experiments may easily be overestimated. It is very difficult to get constant results even with cultures of known pathogenicity. Thus mouse protection tests of antistreptococcic serums have not proven of great significance in judging the value of the serum, because of the inconstancy of control inoculations. The inoculation of animals with a few strains of a group of streptococci might easily lead to erroneous conclusions, but when a large number of organisms of similar properties give constant results some conclusion may be warranted.

The work attempted here was to show whether any pathogenicity was demonstrable in the hemolytic streptococci obtained from milk. Davis (2) reports some pathogenicity for rabbits in a few cultures, but his results are generally negative.

Most of the cultures isolated were inoculated into mice. This was done as soon as possible after isolation. As soon as their purity was assured the organisms were given three daily transfers in serum broth, then 0.5 c.c. of the third broth culture was inoculated into the peritoneal cavity of a mouse. The human pathogens were treated in the same way. All mice were observed daily for seven days and doubtful results were repeated. In no case were any of the typical milk strains pathogenic for mice. One of the "W" strains killed mice and all of them seemed to cause rather severe symptoms. The back would be arched, the hair roughed, and respirations rapid and labored. This seems to confirm the suspicion that these strains are of the pyogenic type and resemble more closely the human pathogenic than the milk strains. All of the former group showed definite pathogenicity for mice (see Tables X and XI). In every case the mice which died

TABLE X.

Results of mouse inoculations with pathogenic cultures.

Culture.	Time after inoculation.				
	18 hours.	24 hours.	36 hours.	7 days.	Septicemia.
24	Normal	Normal	Normal	Normal	
177	Normal	Normal	Normal	Normal	
179	Normal	Normal	Normal	Normal	
180	Normal	Normal	Normal	Normal	
182	Dead				+
200	Dead				+
233	Dead				+
234	Dead				+
238	Sick	Dead			+
240	Dead				+
242	Sick	Dead			+
260	Dead				+
250	Dead				+
252	Dead				+
258	Sick	Dead			+
259	Dead				+
261	Dead				+
247	Sick	Sick	Dead		+
136 (*vir.*)	Normal	Normal	Normal	Normal	
33	Dead				+
7	Sick	Sick	Dead		+
18	Normal	Normal	Normal	Normal	
44	Dead				+
DB	Dead				+
LI (*vir.*)	Sick	Dead			+
Repeated tests					
24	Sick	Dead			+
177	Sick	Dead			+
179	Sick	Sick	Dead		+
180	Sick	Normal	Normal	Normal	
252	Dead				+
234	Dead				+
240	Sick	Dead			+
242	Sick	Sick	Dead		+
186 (*vir.*)	Sick	Normal	Normal	Normal	

TABLE XI.

Results of mouse inoculations with milk cultures.

Culture.	Time after inoculation.				
	18 hours.	24 hours.	36 hours.	7 days.	Septicemia.
U 1	Normal	Normal	Normal	Normal	
U 2	Normal	Normal	Normal	Normal	
U 3	Normal	Normal	Normal	Normal	
U 4	Normal	Normal	Normal	Normal	
F 7	Normal	Normal	Normal	Normal	
F 8	Normal	Normal	Normal	Normal	
F 9	Normal	Normal	Normal	Normal	
F10	Normal	Normal	Normal	Normal	
W 1	Sick	Sick	Sick	Normal	
W 2	Sick	Sick	Sick	Normal	
W 3	Sick	Sick	Sick	Normal	
W 4	Sick	Sick	Normal	Normal	
W 5	Sick	Sick	Sick	Normal	
W 6	Dead				+
W 8	Normal	Normal	Normal	Normal	
W 9	Sick	Sick	Sick	Normal	
C 7	Normal	Normal	Normal	Normal	
C 8	Normal	Normal	Normal	Normal	
C 9	Normal	Normal	Normal	Normal	
C10	Normal	Normal	Normal	Normal	

were autopsied and the heart blood and peritoneal fluid streaked on blood agar plates and inoculated into broth. The organisms thus isolated were always identical morphologically and in their hemolytic properties with those injected. It would seem then that the milk strains were not pathogenic for mice. A number of them were injected intravenously into rabbits with entirely negative results.

Heineman (12) reported that S. lacticus could be made virulent for rabbits by successive subcutaneous inoculations. The organism was re-isolated from the site of inoculation and re-injected into another rabbit and so on. The lesions were said to become more severe until a culture was obtained, after several transfers, which killed rabbits. Although the culture was examined and identified after each isolation, the danger of involving a streptococcus from the body of the animal is not excluded.

Typical milk strains of hemolytic streptococci were injected successively into six mice. The mouse was etherized, in each case, two days after the inoculation and the streptococci isolated from the peritoneal fluid, and occasionally from the heart blood. These were re-injected into the next mouse and so on. One-half c.c. of broth culture was injected into each mouse. The organisms showed no signs of increasing in virulence, the mice in all cases appearing normal. It does not seem possible, therefore, to render a strain of the hemolytic streptococci from milk virulent by passage through mice.

THERMAL DEATH RATES.

The statement is made by Ayers, Johnson, and Davis (13) that pathogenic streptococci are killed at 60° C., when held for 30 minutes in milk and that if any survive this heating, they are so attenuated as not to be dangerous. Some of their cultures which survived this temperature, although coming from pathological sources, were not considered pathogenic. The question is thus raised as to whether or not there is a decided difference in the thermal death rate of the pathogenic and non-pathogenic streptococci. If true, this would settle any doubts in regard to efficiency of pasteurization with respect to streptococci, and furthermore, might offer a convenient means of differentiation between the pathogenic and non-pathogenic streptococci in milk.

Some recent publications have emphasized the fallacy of speaking of thermal death points when all of the factors governing the complete sterilization of the culture are not controlled. It has been repeatedly demonstrated that the time required to kill all the organisms at a given temperature is dependent on the initial number present. According to Phelps (14), "the rate of dying whether under the influence of heat, cold, or chemical poison, is unfailingly found to follow the logarithmic curve of the velocity law, if the temperature be constant." This curve never reaches a zero value, although approaching it indefinitely. He further states that thermal death points "are merely accidental, depending in the first place wholly on the technique adopted and again upon the chance of the last few survivors living or dying, when for a considerable length of time these chances are about equal."

Any method, therefore, which is designed to give a refined comparison between the effect of heat upon different cultures must take into consideration the initial number of organisms and compare, not the temperature at which all organisms are killed in a given time, but the rate of death at a given constant temperature. In other words, the velocity coefficients of the rates of death of the organisms should be compared. Bruett (15) used these velocity coefficients, or K values, determined for spore bearing organisms, before and after "cold shock," in order to determine the utility of "blanching" in food canning. This method made possible a refined comparison of the rates of death of cultures of the same species when differently treated. Differences in heat resistance between the various groups of streptococci ought, therefore, to be demonstrable by this method. It is conceded that the determination of the thermal death point will show differences in heat resistance among streptococci, but in this determination only complete sterilization is considered. The accidental survival of a single or a few organisms is not distinguished from the survival of all or a large proportion of the initial number and thus distinct differences in behavior of two organisms at a given temperature may be obscured.

It has been sufficiently demonstrated that bacteria when subjected to fixed unfavorable conditions, such as a constant high temperature, will die off in a manner corresponding to the law of monomolecular reactions. This law is expressed by the equation:

$$K = \frac{1}{t} \log \frac{B}{b}$$

in which K is a constant, the velocity coefficient of the rate of death,

t is the time between determination of number of organisms.

B is the initial number of bacteria in any time interval.

b is the number of bacteria at the end of time t.

In this formula K varies only with the temperature, is independent of the initial number of organisms, and does not involve complete sterilization. The point of complete sterilization is indefinite, therefore the K is more accurately determined when b is not near the point of complete sterilization. The best results are obtained, according to Phelps (53), when there is about a 50 per cent. reduction in the number of organisms.

It was thought best to determine the velocity coefficients of the rate of death of the hemolytic streptococci in milk, since the application of the results to pasteurization as well as to the differentiation of the organisms, was sought. Each strain was grown in milk for three days, transfer being made each day. The third 24-hour culture was used for the test. The heating was done in a De Khotinsky electric bath in which the temperature was controlled very accurately. A tube containing ten c.c. of sterile milk was placed in the bath and held for five minutes. This was found sufficient to raise the milk to the temperature of the bath. The organisms were then pipetted into the tube from the 24-hour culture. It was found that with the milk cultures one tenth of a cubic centimeter of the 24-hour culture, added to the heated milk tube, gave a suspension containing between one and ten million bacteria per c.c. With the pathogenic strains one c.c. of the 24-hour culture gave about the same number. The latter, therefore, did not seem to develop so abundantly in milk. The difference in counts, however, may have been due in part to the presence of longer chains in the pathogenic strains, with the result that more individuals formed the seed of a single colony.

After the organisms were added the tube was shaken and one c.c. removed for the initial count. The milk strains were enumerated in dextrose infusion agar, but it was found necessary to use whole blood agar to count the pathogenic strains. The colonies were so small on the infusion medium that there was danger of overlooking some of them, while on blood agar the zones of hemolysis around each colony made counting easy. Four plates were made for each count. Two dilutions were plated, each one in duplicate. Counts which did not check satisfactorily, both in the two dilutions and in the duplicates of each, were discarded and the determination repeated.

The tubes while being heated were kept immersed to a point at least two inches above the level of the milk. Before each count the milk was mixed by rolling the tube vigorously between the palms, as far as possible without removing the tube from the water bath. Care was taken to avoid splashing the milk high up on the walls of the tube or on the cotton plug, as in this way some of the organisms might escape being heated to the required temperature.

The test of the applicability of the law of monomolecular reactions to this work is the constancy of the K values at a given temperature. A number of preliminary determinations were made at seven and one half minute intervals with a group of cultures, in order to test the constancy of the K values. The results are best shown by graphs. If the logarithms of the numbers of organisms determined at various intervals, in a culture held at constant temperature, are plotted against time, a line may be drawn, the slope of which is dependent upon K. If the K values are constant for determinations at all intervals of time, the organisms are dying at a constant rate, and the plot should therefore be a straight line. By plotting the logarithms of the numbers, we may test the constancy of K without determining it. Graphs plotted from some of the data are shown in Fig. 1.

It will be seen that the K values do not vary greatly, as most of the plots approximate a straight line. Subsequent determinations with other cultures did not always yield such uniform results, but the variations from straight lines are quite similar in all and can be explained on the same basis.

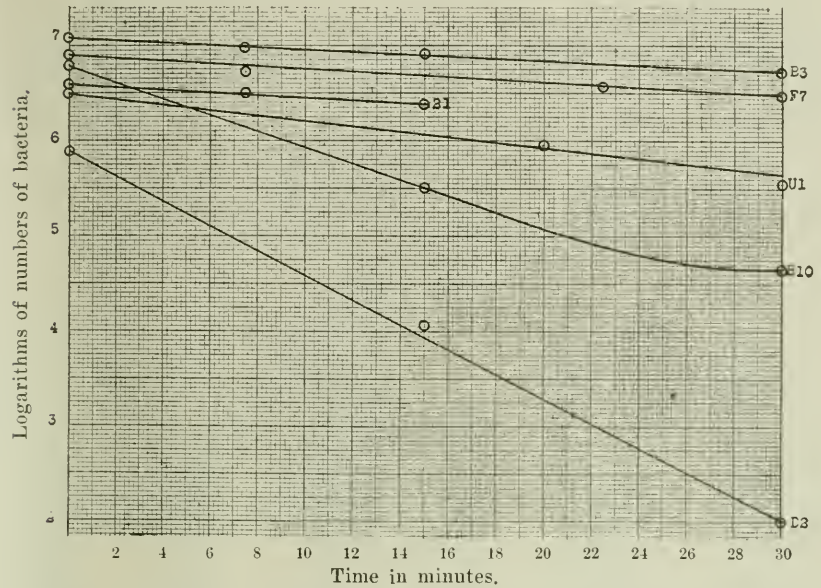

FIG. 1. Graphs showing thermal death rates of hemolytic streptococci in milk at 60° C.

It frequently occurs in these that the velocity coefficient of the rate of death, during the first interval of time, is not as great as in the second and third, also that as the number of organisms gets very small there is considerable variation in the coefficient. The most constant results are obtained when the decrease in the number of organisms does not go much beyond fifty per cent. This may be due to the greater percentage of experimental error in counting small numbers of organisms, or perhaps the law is no longer strictly operative when small numbers are reached.

The slow rate in the first interval of time has two possible explanations. First, a slight error is introduced by the addition of the suspension of the organisms to the heated milk, causing a slight reduction in temperature. The first counts were made as soon after the introduction of the organisms, as the heated milk suspension could be properly mixed. In this way, the sample may have been taken occasionally before the proper temperature was regained. Second, shaking after the first heating interval may cause the breaking up of chains, making a larger number of colonies for the actual number of bacteria present than appeared in the first count.

Although the K's are not constant for all intervals of time, it was considered that the best comparison of the rates of death of two groups of cultures could be obtained by comparing the K's computed for the longest practicable length of time. Most of the K's for the milk strains were, therefore, computed for 30 minutes and those of the pathogenic strains for a ten minute period.

It becomes apparent from an examination of the data in Tables XII–XIII that there is a very marked difference between the behavior of the human and milk hemolytic streptococci at 60° C. The human strains die very rapidly and reach a very small number per c.c. in ten minutes, while the milk strains die very slowly at this temperature; some, in fact, appear not to decrease to any appreciable extent in thirty minutes. It is questionable, because of the small number of organisms left after the time interval, whether or not

TABLE XII.

Rates of death of milk cultures in milk at 60° C.

Culture.	Initial number of bacteria per c.c.	Number of bacteria per c.c. after			
		10 minutes	20 minutes	30 minutes.	K.
U 1	9,700,000	....	....	1,000,000	.0329
U 2	3,300,000	2,100,000	930,000	344,000	.0327
F 7	8,450,000	7,550,000	6,600,000	5,170,000	.0071
U 5	9,900,000	9,800,000	9,900,000	9,800,000	.0614
U 4	11,260,000	11,800,000	....	10,100,000	.0014
F 8	5,200,000	4,300,000	3,800,000	3,300,000	.0056
F 9	4,400,000	4,400,000	3,900,000	3,400,000	.0036
C 7	4,300,000	4,200,000	4,000,000	5,900,000	.0014
C 8	9,000,000	140,000	90,000	20,000	.0884
C 9	7,100,000	2,900,000	1,600,000	1,000,000	.0270
C10	7,000,000	2,900,000	1,390,000	....	.0651
F11	5,600,000	1,600,000	180,000	25,000	.0783
F12	4,500,000	4,200,000	3,400,000	2,700,000	.0073
W 4	3,900,000	27	....	....	.5139
W 6	2,500,000	240	....	....	.4017
W 8	36,300,000	86	....	....	.5628
W 2	4,100,000	0	....	....	....
W 3	530,000	110	....	....	.3662
W 9	3,400,000	107	....	....	.4201

the *K* values for the pathogenic streptococci represent the rapidity of the rate of decrease. The curve of decline of numbers probably reaches a point, before the ten minutes have elapsed, where death is no longer in strict accordance with the law of monomolecular reactions, or in other words, reaches that small number in which chance plays an important part in survival. The determination of the *K*'s, however, obviously gives a better picture of the behavior of the organisms than a determination of the point of complete sterilization—the indeterminable point at which all of these few remaining organisms have died.

TABLE XIII.

Rates of death of pathogenic cultures in milk at 60° C.

Culture.	Initial number of bacteria per c.c.	Bacteria per c.c. after ten minutes.	K.
177	3,000,000	100	.5221
250	5,200,000	60	.4957
258	4,300,000	191	.3400
182	610,000	47	.3257
230	1,460,000	11,400	.2038
259	5,000,000	540	.3986
100	480,000	0	
261	3,900,000	310	.4099
242	11,500,000	266	.4637
254	360,000	3,500	.2011
253	6,600,000	56	.5192
240	300,000	345	.2939
179	765,000	53	.4159
260	4,700,000	101	.4667
247	900,000	16	.4750
252	255,000	2,500	.2008
24	930,000	14	.4822
200	3,200,000	24	.5124

The rates of death of the pathogenic strains are fairly uniform as compared with the milk strains although none of the typical strains of either have a death rate which approaches that of the other group. The "W" strains, as in previous tests, show great similarity to the pathogenic strains. In all of the tests they have been identical with the pathogenic group and there seems to be little reason to believe that they are not of this group. None of these organisms were obtained from pasteurized milk but from milk produced under the best sanitary precautions. It would be difficult to prove whether or not these strains could be pathogenic to man, but their similarity to pathogenic strains and the fact that they were found only in unpasteurized milk, argues very strongly for pasteurization of all milk regardless of the care used in producing it.

The great difference in the thermal death rate of pathogenic human and non-pathogenic milk streptococci suggests that this character might be added to the list of characters which serve to differentiate them. It is one which can easily be determined with simple apparatus. It is also a character which perhaps reflects the habitual environment of the organism, that is, a purely saprophytic organism would be more likely to withstand wide ranges of temperature than one which lives and grows largely at the temperature of the human or other animal body.

The question as to whether pathogenic streptococci might survive pasteurization arose in connection with these experiments. The usual pasteurizing temperature is 145° F. or 62.8° C., although 60° C. is occasionally used. Ayers, Johnson, and Davis (13) concluded that pathogenic streptococci will not survive this temperature for 30 minutes.

All of the pathogenic strains used in the above tests were held at 60° C. in milk for 30 minutes. All strains were tested several times. The tubes were cooled after heating and incubated for 48 hours at 37° C. Occasionally some of them survived, grew in the milk, and were carefully identified. In these tests the initial number of organisms was the same as that in the determination of the death rate, that is, 1 c.c. of a 24-hour milk culture was added to a tube containing about 10 c.c. of milk at 60° C. Some of the cultures which grew after the one heat test failed to grow after a second test. Others, namely, 33, 44, DB, and LI (S. viridans), grew quite consistently after heating for 30 minutes at 60° C. If the initial number of organisms was reduced to 0.1 c.c. of a 24-hour milk culture, none of the cultures survived the test.

The question arose whether the organisms lost their virulence by the heating. To test this, hemolytic culture No. 33 and viridans culture LI were inoculated into mice. Both mice died in 24 hours. The organisms were isolated from the heart blood on blood agar plates and identified. A single colony of each was transferred to a milk tube and incubated for 24 hours. One c.c. was transferred from each of the tubes to sterile milk tubes at 60° C. and held for 30 minutes. The tubes were then cooled and incubated for 24 hours. 0.5 c.c. of each was injected intraperitoneally into a mouse and a third mouse was inoculated with 0.5 c.c. of sterile milk. Both of the mice, inoculated with the heated streptococci cultures, died within 48 hours, while the control survived. The organisms were again isolated from the heart blood and identified. This simple experiment seems to show that the virulence of the streptococci which survive pasteurization is not lost, or at least they are able to produce virulent cells in the same milk in which they were heated. The experiment was repeated with the same results.

It must be remembered that in these experiments the number of organisms present was very large. There were probably from one to five million bacteria per c.c. present at the beginning. One would scarcely expect to find milk so highly contaminated with pathogenic streptococci. However, the margin of safety in pasteurization would be much more adequate if higher pasteurizing temperature were used.

It was thought that repeated heating of a culture might lead to the selection of a heat resistant strain of the organisms. Accordingly two hemolytic cultures, No. 33 and DB, and viridans culture LI were pasteurized at 60° C. for 30 minutes, every second day for a time. It was intended to heat them ten times and then to determine their death rates and compare them with those of the original cultures to see if the heat resistance of the organisms had increased. None of the three cultures, however, survived ten heatings; the viridans strain was killed by the sixth heating, the hemolytic strain No. 33, by the eighth, and DB by the ninth. It seems clear that the cultures did not gain in heat resistance by the repeated heatings. The apparent decrease in resistance after a number of heatings could not be explained.

CONCLUSIONS.

The number of cultures used in this work was not great, especially the group of human pathogenic cultures, yet they were carefully selected and are believed to be fairly representative. Several conclusions may, therefore, be warranted from the study of the data presented:

1. Hemolytic streptococci which differ in many respects from human pathogenic streptococci occur quite generally in raw and pasteurized milk. A large number of these strains behaved alike in a series of tests, and they therefore seem to form a homogeneous group or species.

2. There are certain general differences in morphology between milk and human hemolytic streptococci. These are not absolute and by themselves are of little value for purposes of differentiation.

3. The reaction of milk hemolytic streptococci in litmus milk is

characteristic and is of importance in differentiating them from pathogenic strains.

4. The pathogenic hemolytic streptococci have a greater range of fermenting power than do the milk strains. The most constant difference between the two groups is the failure of the latter to ferment saccharose.

5. The identification of hemolytic streptococci should always be confirmed by testing for hemolysis in broth culture. The true Beta type produces hemolysin in broth, while others which resemble the Beta type quite closely do not.

6. There is no constant difference in the hemolytic titer of milk and pathogenic strains, but the time required for hemolysis to take place is distinctly greater with the milk strains. This characteristic could be used to aid in differentiation.

7. The milk hemolytic streptococci are not pathogenic for mice, and virulence cannot be demonstrated even by successive passages.

8. The difference in the thermal death rates of milk and pathogenic hemolytic streptococci serves to differentiate them. The former die at a slow rate in milk at 60° C., while the latter die rapidly.

9. With a very large number present it is possible for pathogenic streptococci to survive pasteurization at 60° C. for 30 minutes. The number of survivors is undoubtedly very small, but they are able to produce a culture virulent for mice. Higher pasteurizing temperatures would give a better margin of safety.

10. Successive heating of pathogenic streptococci does not result in the production of a heat resistant strain.

11. Hemolytic streptococci resembling the pathogenic type in every respect were isolated from certified milk. They are of low heat resistance and could readily be destroyed by proper pasteurization.

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THE PROBLEM OF PLAGUE IN THE UNITED STATES.

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Bubonic plague has been a sanitary problem in the United States for the last two decades, as the first cases which ever occurred in North America were recognized in San Francisco in 1900, the infection having come from Honolulu where cases had been occurring for some months, or from a more distant Pacific port.

The early outbreak on the Pacific coast was confined chiefly to Orientals in the Chinese quarters of communities, and it is one of the many unexplained features of the epidemiology of the disease that it did not become fairly prevalent at that time among other elements of the population, since, a few years later when it recurred the white population furnished the large majority of the victims, and the Orientals very generally escaped the infection. We can scarcely attribute the restriction to one racial group of the population in the early outbreak to the eradicative measures employed, as the epidemic occurred prior to the time when we knew anything definitely helpful of the relation of rodents to the disease, and consequently methods of control were not, in the light of our present knowledge, very effective.

The disease was confined to the Pacific coast and to a few localities there until 1914 when it appeared in New Orleans where it was dealt with in vigorous and efficient fashion. The last case in this outbreak occurred in 1915, and during a period of four years no cases were reported in the eastern section of the country. In the autumn of 1919 plague again appeared in New Orleans and a few months later cases occurred almost simultaneously in several other Gulf Coast cities.

While on the mainland the rat epizootics as well as outbreaks of plague in man have been controlled within reasonable periods, in one of our insular possessions the rat infection has been most persistent. On the northern coast of the island of Hawaii in the Hawaiian Islands, in a highly developed agricultural region, rats in the sugar-cane fields have been found infected. In this particular section plague has occurred among people and among rats from time to time for about twenty years. During this period there have been intervals of two years or more between human cases, and a year or more between rodent cases, but the infection has never been eradicated though well-directed efforts have been made to exterminate it.

The type of the disease in the United States and its possessions has always been that characterized by primary involvement of the lymphatic structures—in other words, the bubonic type of plague, except in a circumscribed outbreak of the pneumonic form which occurred in California in the autumn of 1919, and which will be considered later.

We have now had sufficient experience with plague in this country to justify a survey of the problem, and we may safely say that the subject may be viewed in a much more optimistic light than would have been possible a few years ago. We have learned that under conditions such as are found even in our communities which do not have very high sanitary standards the disease does not become a disastrous epidemic such as we feared might follow the introduction of the infection before our own experience with it. The deaths from plague have nowhere in the country been sufficiently numerous to be reflected seriously in mortality statistics.

Fortunately, the time is past when it is either popular or expedient to conceal the fact of the presence of plague in a community or to dispute the diagnosis in the interest of local commercial advantages. It is to be regretted that equally full data are not promptly forthcoming on the prevalence of the disease in some European countries.

SEASONAL PREVALENCE.

The outbreaks appear to show a fairly well-defined seasonal predilection as shown by the following tables covering mainland outbreaks, the summer and autumn months yielding the great majority of cases.

In Porto Rico the single outbreak reported on that island, consisting of 56 cases, occurred during June, July, August and September of 1912; In Hawaii, on the other hand, the two chief outbreaks occurred in December, 1899, and January and February, 1900, when 80 cases occurred, and in April and May of 1907, when 34 cases occurred. Obviously, among other factors which must be con-

PLAGUE IN MAN.

(Rat origin.)

PACIFIC COAST.

	1900	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	Total.
January					3				1					4
February		8	1		5				1					15
March	3			1	1									5
April	1	2	1											4
May	6		3					1						10
June	3			1										4
July		3	4	4										11
August	2	1	9	2				11						25
September		6	7	1				41						55
October	4	4	6	5				44						63
November	2	1	3	3				37						46
December	1	1	1					36						39

LOUISIANA.

	1914	'15	'16	'17	'18	'19	'20	Total.
January								
February								
March								
April								
May							2	2
June	3						1	4
July	11							11
August	9	1					4	14
September	7							7
October						3		3
November						5		5
December						4		4

TEXAS.

	1914	'15	'16	'17	'18	'19	'20	Total.
January								
February								
March								
April								
May								
June							10	10
July							5	5
August							9	9
September							4	4
October							3	3
November								
December								

FLORIDA.

	1914	'15	'16	'17	'18	'19	'20	Total.
January								
February								
March								
April								
May								
June							4	4
July							3	3
August							2	2
September							1	1
October								
November								
December								

(*Squirrel origin*).

CALIFORNIA.

	1904	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17	'18	'19	'20	Total.
January															
February	1														1
March															
April														1	1
May															
June				1			1								2
July		2			1				1						4
August		2		1	1										4
September		1			1		1						1		4
October			1												1
November															
December															

sidered in connection with seasonal prevalence are the time of introduction and the limitations placed on the outbreaks by activities designed to suppress the infection, and the influence of these cannot be accurately estimated now.

BACTERIOLOGY AND PATHOLOGY.

This is not the place to discuss at length the bacteriology and pathology of plague, but it may be emphasized that the organism is one readily recognized by cultural and animal tests; indeed, there are no organisms likely to be gotten from human sources which cause any confusion. From lower animals we may obtain several organisms which need to be differentiated. These are Pfeiffer's pseudo-tubercle bacillus of rodents, and certain organisms of the hemorrhagic septicaemia group, but these never occur as natural infections in rats.

The gross pathologic features of the disease in man and in animals are as characteristic as are the biological characteristics of the organism. Cases, either in rats or in man, are exceptional in which the post-mortem examination leaves one who is familiar with the disease in any serious doubt as to the diagnosis.

Some unwarranted confusion has been occasioned by the plague-like disease of rodents which is found naturally in California in ground squirrels, in the middle West and in Utah in rabbits, and in Utah in "quimps," a squirrel-like rodent. The disease, in naturally-infected rodents and in those artificially infected, particularly guinea pigs, induces lesions which will often defy differentiation from plague, even by a person thoroughly familiar with both plague and the plague-like disease. The causative organism of the latter, viz., *B. tularense,* bears no resemblance in any respect to the plague bacillus. It has the very striking and characteristic feature of growing only on egg media, or on a medium containing egg yolk.

RODENT CARRIERS.

In this country plague has been found naturally in the following rodents of our cities: the gray rat (*Mus norvegicus*), the black rat (*Mus rattus*), the white-bellied rat (*Mus alexandrinus*) and the mouse (*Mus musculus*).

The three varieties of rats are found in varying proportions in the several communities of the United States in which rat surveys have been made. The gray rat predominates throughout the country as a whole, but in southern ports the black rat, including the white-bellied variety, forms a large percentage of the rodent population; indeed, it is the predominant species in some cities. These three rodents are all concerned in the transmission of plague, though it would be unsafe to attempt to indicate their relative importance.

We know little of the relation of mice to plague, either in rodents or in men. They have been considered as of little importance, because they are regarded as not migratory, but their relation to epizootics and epidemics needs further study.

We do not have convincing evidence that the epizootic and the epidemic always run simultaneously; indeed, in some of our outbreaks the crest of the detected rodent cases followed by a considerable period that of plague in man, and again, especially at the decline of the epidemic, human cases occurred so far separated from any detected rodent plague as to leave the association impossible of establishment. The infection of rodents may be very light; thus, in one year but a single infected rat was found among over 148,000 taken in Hawaii, though probably not all of the rats were examined. In another year, among about 122,000 rats taken but two were found to be infected.

One of the most important questions in regard to rat plague is the significance of subacute or chronic plague in rats. This form of infection was not found in San Francisco but it appears to be relatively common in our Southern cities. The serious question as to whether chronic infection serves as a starting point for new outbreaks of the acute form remains unanswered. We do not know whether the outbreaks in our Southern ports during the past year were caused by a recrudescence of the old infection, due to subacute rat plague in New Orleans, or to a new importation.

Among our rural rodents or among those usually regarded as city dwellers but sometimes living under rural conditions we have had natural infection among the ground squirrels (*Citellus beechyii*) of California, the black rats (*Mus rattus*) of Hawaii, one species of wood rat (*Neotoma*) and a field rodent in Louisiana. Squirrel plague has been studied intensively for over ten years, and we know that plague in these rodents presents some rather unusual features as compared with the disease in rats. In a large number of cases the disease tends to assume a subacute or chronic form, and doubtless many infected squirrels recover. The lesions induced in guinea pigs by artificial infection from squirrels also tend to be subacute. Furthermore, though the data on this point are not extensive, there is some evidence that human infections due to squirrels are less fatal than those of rat origin, and the clinical picture may be that of a subacute process more often than occurs in cases infected from rats.

The human cases traceable to squirrels almost always have the peculiarity of having the primary buboes in the axilla, due, of course to the fact that in the handling of infected squirrels, dead or alive, the fleas attack the ''upper extremities'' while, when the disease is contracted from rats, the chances are that infection will be through the skin of the feet or legs, resulting in primary buboes in the femoral region.

Squirrel infections account for very few human cases; only about 17 in all have been found since the squirrel origin of plague has been established; indeed, at present human infections from squirrels occur so rarely, not averaging more than one per year, that this feature may be regarded as relatively negligible.

The serious aspect of the squirrel plague problem lies in the danger of the disease being transmitted to the rats of urban communities and leading to recurrence of rat plague, or to infection of localities not heretofore infected. With this risk in mind, about ten years ago there was inaugurated the work of making a squirrel-free zone around the large cities of San Francisco Bay, with a view to protecting the rats of these communities from infection by squirrels in the adjacent regions which showed a high incidence of plague.

The magnitude of the problem of exterminating squirrel plague cannot be adequately appreciated without realizing that the infection exists in an area at least 150 miles long by 50 miles wide. There have never been sufficient funds available to enable sanitary authorities to deal adequately with it, and the plan of dealing with it through legislative mandate to owners of property which harbors squirrels has not proven effective.

It is indeed a serious question whether it is economically justifiable to attempt the eradication of squirrel plague, desirable as it may be from some points of view.

INSECT CARRIERS.

While any blood-sucking insect may take up plague bacilli from the blood of human or rodent cases, epidemiological data seem to justify us in giving serious attention to fleas only.

Flea surveys in San Francisco show the *Ceratophyllus fasciatus* to be the common rat flea, with the *Loemopsylla cheopis* forming only a small percentage of the total; on the other hand, in the Southern ports the proportions are reversed. Both of these attack man, and we know that experimentally either of these may be a satisfactory medium for plague infection. The common mouse flea, *Ctenophthalmus musculi,* stands alone among common rodent fleas as one that refuses to attack man.

The common squirrel flea, *Ceratophyllus acutus,* has been shown to be capable of carrying plague among squirrels and from the latter to other rodents.

It has been shown by English workers that the transfer of plague from animal to animal by fleas is not so simple as was believed at one time. It appears that multiplication of the bacilli takes place in the flea's stomach, with some obstruction of the blood-sucking apparatus which leads to regurgitation and injection of a heavy suspension of the infecting organism.

Plague has been transmitted experimentally among rodents by other insects; bedbugs and lice have been found to harbor the organism, but there is no evidence to connect any of these in a significant way with naturally acquired rodent plague, or with human plague.

Interurban spread.

While we are in possession of fairly satisfactory information as to the mode of spread of plague in communities, its mode of transfer from one community to another is not always clear. That the disease is usually borne by water-carried commerce is attested by the fact that seaport cities are most likely to be infected; thus, New Orleans, Galveston, Beaumont, and Pensacolo have been our only active foci in the past year, and these are all Gulf ports. But, on the other hand, Mobile, Key West and New York have escaped, though they have more communication with infected ports than do Pensacola or Beaumont. While there is considerable water traffic between New Orleans and the cities northward on the Mississippi River, as well as much railroad traffic, none of the latter cities have shown evidence of infection.

Carrying of plague in rats by railway freight traffic doubtless occurs; indeed, it is often impossible to decide between the possibility of railway and steamer transfer of infection. Regardless of the precise means of carriage, all evidence points to rats as the agents by which infection is borne from one place to another.

Suppression.

The measures at our disposal for combating urban plague are those which make for reduction of the rat population. Eradication of these rodents is not to be considered as in the realm of the practicable, but it is probable that a sufficient reduction in numbers can be effected to make difficult the perpetuation of the infection among them. One officer experienced in plague work has expressed the opinion that when the rat population had been reduced by about 50 per cent. rodent and human cases seem to cease.

There is much room for improvement in our methods of rat destruction, but we have at hand traps and poisons which are fairly effective, and of course proper disposal of edible garbage discourages rat harboring. Natural enemies of the rat, be they domestic animals such as cats and dogs, or wild ones such as the mongoose, have no appreciable influence on the rat population under ordinary conditions.

A discussion of this part of the subject will be completed by a reference to "Rat Viruses." Efforts to start an epizootic among rats or mice that should wipe out or greatly reduce these rodents, and yet not be a menace to other animals or to man, have met with scant success so far as domestic rodents are concerned. The writer has tried a number of the viruses and while they will kill a large percentage of the rats to which they are fed, we have seen no considerable spread of the infection to rats in contact with those infected with the virus, or in those which devour the bodies of the rats dead of the infection.

Rat proofing of buildings and docks, from which so much has been hoped, is of great value, but the difficulties of securing the general adoption of the measure are very great.

Some who have had an opportunity to observe plague and measures for its eradication in various communities in the United States believe that it is not always an evil unmixed with blessings. Much permanent good has attended rat eradicative measures. I refer especially to improvement in garbage disposal facilities and improved sanitation of dwellings. In a commercial way it has been advantageous; for example, rat-proof docks, though expensive to construct, will be economical in the end, not only by reducing loss from rodent depredation, but by lessened cost of upkeep, and the same applies to certain warehouses.

Rat surveys.

Our large cities, especially those having water communication with plague-infected ports, should carry on systematic and sustained examinations of their rat population in order that plague, if introduced, may be detected promptly, so that radical measures of suppression may be taken. If this is done we may properly entertain the hope that a toll of human lives may be avoided.

It would be hazardous to specify the number of rats that should be examined in such a survey, but 1,000 rats per month for each 100,000 of the human population ought to give information of the presence of the disease among the rodent population early enough to enable the sanitary authorities to take appropriate measures for its suppression. The water-front, railway yards and warehouses adjacent thereto are the locations to which most attention should be given.

Earnest pleas have been made for rodent eradication on the ground that rats, mice and squirrels destroy food and damage merchandise and other materials, and that they are expensive parasites. While generalizations as to the economic destruction wrought by rats probably are untrustworthy, still the loss is by no means insignificant, but so far as domestic rats are concerned, and they are the ones which concern us from a public health point of view, the propaganda for eradication has not been taken seriously enough to lead to any determined effort to reduce rodent population.

IMMUNITY.

The whole field of *plague immunity,* including serum therapy, needs much further study before it can be regarded as being on a satisfactory basis. When we realize that a serum having high protective power experimentally may fail to influence cases in any manner clinically, we understand the urgent need for further study of the subject. The serum, though always to be used, frequently disappoints, and the plague vaccine is of uncertain value in epidemic work.

PNEUMONIC PLAGUE.

With the exception of a single group of cases we have thus far been free from primary *pneumonic plague* in this country. An outbreak, consisting of a total of 13 cases, arising from a bubonic case of squirrel origin which developed secondary pneumonia that spread through four generations in man, occurred in California in the autumn of 1919. Pneumonic plague in man rarely occurs from rat infections, and it is an interesting and possibly significant fact that in plague squirrels there is a very definite tendency to pulmonary localization, a condition which never occurs in plague in rats.

The whole subject of the relation of pneumonic to bubonic plague is very obscure. In many respects they are so dissimilar as to make it difficult to realize that the two diseases, clinically so different, are due to the same organism, and that the cultures from each type of cases are not to be distinguished from those derived from the other type.

THE RELATION OF CERTAIN CARRIER CONDITIONS TO THE VIRULENCE OF HEMOLYTIC STREPTOCOCCI.*

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It has been demonstrated by a number of observers that the hemolytic streptococcus is a common inhabitant of the tonsils, especially of the crypts. Smillie (1917) found a large percentage of healthy carriers in a boys' school during an epidemic of sore throat. During the past few years numerous instances of a high incidence of healthy carriage of this organism in army camps have been reported. Nichols and Bryan (1918) report observations on the treatment of chronic carriers. They state that inasmuch as the hemolytic streptococcus appears to have a special predilection for the crypts of the tonsils rather than the surfaces of these and neighboring structures, it is especially difficult to treat carriers of this organism successfully. Their conclusion is that extirpation is the only satisfactory method. Davis (1920) states that the crypts are an almost constant source of hemolytic streptococci.

Studies of extirpated tonsils thus have added to our knowledge of carrier conditions. Since the crypts, in a large number of instances, contain hemolytic streptococci among their apparently normal flora, cultures of the tonsil surfaces and the pharynx, unless carefully made, will fail to reveal true carriers of this organism. Also it consequently appears that carriers of this organism are more common than formerly supposed.

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Altho reports of the incidence of carriers show these facts, studies of the relative virulence of the organisms carried and the relation of the acuteness or chronicity of the carrier state to the virulence of the strain harbored have not attracted much attention. It is a commonly observed fact that as the number of healthy carriers of any pathogenic organism increases in a group or community of individuals an epidemic is to be feared. This has been explained not only for the reason that there has been an increased opportunity for infection but that the virulence has been increased by more or less rapid passage from one individual to another.

The results of this report embody experiments dealing with the relative virulence for mice of strains of hemolytic streptococci obtained from different groups of healthy carriers, strains obtained from excised tonsils and strains from acute respiratory infections.

Source of cultures.

Cultures were obtained from the following groups: (1) chronic carriers, i.e., individuals who had yielded positive cultures of hemolytic streptococcus from their tonsils or pharynx at different intervals for a period of at least three months; (2) early carriers, individuals who had harbored the organisms for only a few days or weeks, and without history of prior infection; (3) cases of incipient acute bronchitis, laryngitis or tonsillitis; (4) cultures from tonsils removed several months following an attack of tonsillitis.

Technic.

In obtaining the first three groups of cultures, the tonsils and pharynx were swabbed and the material streaked on blood agar plates, in the usual manner. Colonies were picked after 18–24 hours incubation and transferred to blood agar slants. In obtaining cultures from the extirpated tonsils cultures were made immediately after removal, both of the surfaces and of the crypts. To obtain the latter the tonsil was cut in half with sterile instruments and material obtained from several crypts. Both tonsils from each case were cultured in this way. It was usually found that the results were the same; occasionally, however, one tonsil yielded hemolytic streptococcus while the other would be negative.

For the determination of the virulence of a given strain the first blood agar slant culture, obtained from the streaked plate, was used.

One fifth of an 18–24-hour growth of such a slant, suspended in salt solution, was injected intraperitoneally into a mouse. Using an amount of culture of this size, the strain was not considered virulent unless the mouse died in 24 hours.

The number of positive cultures obtained from the extirpated tonsils, three to six months following the acute inflammation, is interetsing. As is seen from Table I 96 per cent. of the 75 cases gave positive cultures, a finding in keeping with previous results (3). Furthermore, in over 93 per cent., in all but five of the seventy-five, hemolytic streptococci were present in large numbers, predominating over other organisms present. In two of the negative cases a hemolytic staphylococcus was present as the predominating organism.

TABLE I.

Hemolytic streptococci from tonsils removed three to six months following acute attack.

		Per Cent.
Exterior surface predominant	3	4.0
Crypts predominant	31	41.3
Approximately equal numbers—crypts and surface	36	48.0
Present in small numbers (a few colonies only)	2	2.6
Negative	3	4.0
Total	75	

VIRULENCE TESTS.

The following table shows the results of the virulence tests with the different strains obtained.

TABLE II.

Virulence tests.

	Virulent.		Non-virulent.		Total Strains.
	No.	Per cent.	No.	Per cent.	
Early carrier cultures	3	12.5	21	87.5	24
Chronic carrier cultures	4	10.0	37	90.0	41
Extirpated tonsils (3–6 months after acute attack	7	14.5	41	85.5	48
Acute respiratory infections (tonsillitis, laryngitis, bronchitis)	19	86.4	3	13.6	22

DISCUSSION.

The difference in virulence for mice between healthy carrier cultures and strains obtained during an acute infection with hemolytic streptococci, as shown in Table II, is marked. While no conclusions concerning virulence for one species can be drawn from evidence of virulence for another species of animal, yet the parallelism between these groups is sufficiently striking to be worthy of consideration. In view of the size of the inoculation it is interesting to find that only a small minority of the healthy carriers harbored strains that were at all virulent for mice, while in the group of cultures obtained from actual infections, the converse was true.

No differences in the virulence for mice of the strains obtained from early and from chronic carriers were observed. The majority of the early carriers in this particular group were associated with the carriers of longer duration and doubtless harbored much the same strains. Furthermore this group contains only individuals who gave no history of clinical infection. However, a comparison of the two groups seems to point to the fact that the duration of a strain in a healthy normal throat has little to do with its virulence as evidenced by mouse inoculation. This seems to be true for healthy throats.

A comparison of the group of strains obtained from acute respiratory infections with those from removed tonsils shows a general difference in the comparative virulence of each group for mice. The majority of the organisms isolated from the inflamed throats and tonsils, i.e., at the time when there is actual infection, were virulent for mice, usually in much smaller doses than the amount, one fifth of a culture, used in the tests. Yet after the infection has been overcome and the carrier state ensues, a marked decrease in the virulence of the strain apparently takes place. It would seem that during the acute infection, while the host is combatting the organisms, the latter, at least those successful in resisting for a time the defensive mechanism exerted by the host, develop an increased invasive power. Later, when the infection has been overcome, the organisms are living on the surface of the normal mucus membranes in a state of symbiosis, doing no harm, because, in the present state of the host's resistance, actual invasion is impossible. Under these conditions the invasive power—the virulence—of the organisms diminishes.

A further point of interest is brought out by Table I. This table seems to show that a high percentage of cases of tonsillitis become chronic carriers of hemolytic streptococci. Removal of the tonsils seems to be the only way to treat such cases, since the organisms occur deep in the crypts and usually in large numbers. While these strains apparently decrease rapidly in virulence for the individual who harbors them and for mice, it is impossible to assume that these carriers are, therefore, harmless to others.

The decrease in virulence following an attack of tonsillitis may be a partial explanation of the small number of generalized infections following removal of the tonsils from three to six months after the acute attack, as compared with the higher incidence when they are removed soon after the actual infection. The organisms are still present but not only has the individual developed increased resistance; the bacteria, as well, have diminished in invasive power.

Summary.

A low degree of virulence of hemolytic streptocicci for mice is found to occur in cultures taken from healthy carriers, as compared with those from cases of actual infection.

No differences in virulence have been observed between strains from early and from late carrier states. The duration of a strain in a healthy throat seems to have little to do with its virulence.

A rather rapid decrease in virulence seems to occur after the acute infection has ended. Chronic carriers of hemolytic streptococci as a result of an attack of tonsillitis show a very small percentage of virulent strains.

A large percentage of cases of acute tonsillitis become chronic carriers of hemolytic streptococci, as evidenced by cultures of tonsils removed three to six months following the acute attack.

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THE REAL RISK-RATE OF DEATH TO MOTHERS FROM CAUSES CONNECTED WITH CHILDBIRTH.*

By WILLIAM TRAVIS HOWARD, Jr.

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Introduction.

It is a fair statement that few, if any, of those interested officially and unofficially in public health in the cities and states of this country have any really intelligent appreciation of the high fatality risks of pregnancy and of delivery from that state. Seriously thoughtful consideration of these subjects is almost entirely confined to a few leading obstetricians, whose warnings and teachings only too often fall upon deaf ears. With the exception of these and of some of the more intelligent of those engaged in the infant welfare movement who have realized from practical experience, supported at times by figures not often dignifiable by the term statistics, that the reduction of infant mortality is to a large degree bound up with pre-natal, natal, and post-natal care of the mother, few in this country have any accurate conception of the risk run by the average woman in her lying-in. The great movements directed against puerperal infection since Semelweiss' discovery, Holmes' crusade, the exposure of the deadly obstetrical hospitals, the recognition of the bacterial causation of the disease, and the perfection of the anti- and a-septic methods exhausted themselves, after improving obstetrical hospitals, teaching, and nursing, in inadequate regulations of midwives. In the meantime, researches in obstetrics advanced knowledge in regard to the recognition and control of other causes of maternal mortality in the pregnant and lying-in states; such as, hemorrhage, intoxications, malformations and malpositions, rupture of the uterus, and the like, which, taken together, account for the larger moiety of maternal deaths. But, the relatively highly developed knowledge concerning practical obstetrics is so meagerly diffused and so ineffectively applied to practice, that certainly in the registration area of the United States it is not brought to bear with sufficient force to prevent the occurrence of innumerable major and minor illnesses and a large number of unnecessary maternal deaths.

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One of the most important functions of vital statistics is to determine accurately the risk of illness and of death of those actually exposed to a particular disease or disease group, or, in other words, to measure specifically the forces of morbidity and of mortality. The terms of the risks so determined are commonly expressed in ratios or rates indicating the proportions of the numbers affected to the numbers exposed, and the real value of such rates must depend upon the accuracy and the comprehensiveness of the data in regard to both the affected and the exposed. It is only when the data include such essential qualities as age, sex, race stock, and occupation that the resulting rates may be regarded as satisfactorily specific. In actual experience, the data available to vital statisticians are, in general, so lacking in comprehensiveness in these respects, that there exists hardly a single example of an accurately defined rate expressed specifically in terms of risk on the part of those definitely exposed to illness or to death from a particular disease. In consequence, the public health official is seriously handicapped in defining his problems, in devising administrative measures, and in estimating their value. Likewise, the student of the natural history of disease must so often appeal in vain to the statistical method for accurate measurement of the forces of morbidity and mortality and must remain content with rates relatively crude and subject to various adjustments in the process of being rendered specific for even one or two categories. In measuring the force of mortality in affections of the puerperal state, it has been customary to use rates based on the number of maternal deaths in relation to (*a*) the whole population, (*b*) the whole number of females, (*c*) the number of females within the usual childbearing ages (15 to 45 years), or (*d*) the total number of labors as evidenced by the number of reported live births. Of these methods, the latter is obviously the only one that even approaches specificity; the others are, in the nature of things, necessarily so general and so wide of the mark that they are evidently useless for any serious attempt to estimate the force of mortality in a single community, or to compare it either in the same community over a term of years or in different communities. Risk-rates calculated on the number of maternal deaths from causes connected with the puerperal state and the number of live births occurring within a given period are subject to two important sources of error. The number of live births, used here as the divisor, does not accurately measure the actual number of labors, i.e., the number of women exposed to death in childbearing; for some labors produce more than one living child, and stillbirths, which in some cases are dependent upon conditions that materially enhance maternal risks and which represent a variable but not inconsiderable number of labors, are left out of account.

It occurred to me in 1917, when confronted with problems of this character in the Health Department of Baltimore, that by using the sum of the living and of the still births reported and the number of the deaths of mothers ascribed to puerperal affections, annual death rates might be obtained, expressing in specific terms the actual risk of dying for women definitely exposed in childbearing. Stated in formal terms, the true puerperal risk-rate in childbearing would be derived from the formula:

$$RP = \frac{DP}{BL + BS},$$

where DP equals the number of maternal deaths due to causes definitely connected with the puerperal state and BL and BS equal the number of live and still births respectively.

Risk rates calculated on this basis for the total deaths in the puerperal state and for the deaths under the different rubrics 134 to 141, inclusive, of the international classification of the causes of death, separately and in various combinations, on the data of the Baltimore Health Department for 1915 to 1917, gave interesting tables. Owing to inability to find data of sufficient value on which to base comparative studies—the Reports of the Registrar-General of England and Wales and the Birth Reports of the United States Census for the birth registration area being silent on stillbirths—the matter was dropped for the time. Recently, on becoming interested in the subject again, it occurred to me that by utilizing the data of several large American cities, in which it might be assumed that the reports of live and still births and the reporting and classification of deaths are reasonably accurate, figures sufficiently large to eliminate chance and probable errors might be obtained. For this purpose, New York, Philadelphia, Boston, and Baltimore were chosen for the five year period 1915 and 1919, and, as will be pointed out later, there is a remarkably close correspondence in the rates obtained, and the rates calculated from the figures of these cities seem to afford a fairly accurate criterion of the risk-rate of death applying to childbearing in women in these particular urban communities. Fortunately for purposes of comparison of the value of the method and investigation of the whole subject on a broader scale in this country, the report for 1918 of the Bureau of the Census on births within the registration area includes stillbirths, and this large material is now available for the first time for such uses.

Before discussing the results of this inquiry, it is necessary to point out that on looking further into the question, I found that in the idea of taking stillbirths into consideration I was anticipated by Dr. William Farr, at least. Deeply interested in this subject from a date early in his connection with the Registrar-General's Office (discussing this subject first in the Fifth Annual Report, 1843), he dealt with it fully in the Seventeenth Annual Report in 1856, when he utilized the data of the eight year period 1847 to 1854, which he expressed in tables of rates obtained by diving the material deaths by the total number of live born children. But he clearly recognized, that, as the term *Births*, as used in the returns, expressed only the number of children born alive and not the exact number of childbearings or accouchements, to ascertain the true danger of childbirth to mothers, it was necessary to take into account not only the reported live births, but stillbirths and plural births. Correction for plural births on the basis of the returns for 1852 indicated the necessity of a reduction of about 1 per cent. from the number of reported births, and correction (based on some French figures) for stillbirths required the addition of about 3 per cent; so it would appear that if something like 2.5 per cent. be added to the number of reported live births, the sum would indicate with some degree of accuracy the actual number of childbearings. He concluded that "on an average of eight years (1847 to 1854), to every 10,000 children born alive 54 mothers died; so about 53 in every 10,000 or 1 in 189 accouchments were fatal." Farr classed puerperal deaths under metria—the term adopted for puerperal infection in his statistical nosology—and all other causes. In the same article, he presented the following table, showing the mortality per 100,000 childbearings of women of different ages:

TABLE I.

Ages of women.	Total deaths.	Deaths by metria.	Deaths by other accidents.
15–25	668	277	391
25–35	425	148	277
35–45	633	154	479
45–55	883	163	720

Commenting on this table, Farr pointed out that the mortality in childbearing is least during the age period 25 to 35—when more than half of the children are borne by their mothers—and that as the rates of mortality in the last three age periods are in nearly the ascending ratio as 2, 3, and 4, the calamitous deaths of mothers in childbed are governed by a mathematical law. He further estimated that the death rates for women from all causes for these age periods were raised by childbearing risks per 100 as follows:

15–25	.820 to 1.488
25–35	.327 to 1.417
35–45	.535 to 1.831
45–55	.874 to 2.496

In the Thirty-third Annual Report in 1870, Farr, replying to the criticisms of Mathews Duncan, demonstrated that the returns to the Registrar-General were reasonably complete and that the figures used by him included deaths only in childbearing and excluded deaths due to other causes but occurring in pregnant or childbearing women; i.e., that the classification was designed to show the actual causes of death. Among other things, Farr showed that in eleven large English towns the childbearing mortality was 4.9 and in sixty-four healthy country districts, 4.3 per 1,000.

Returning again to the subject, in the Thirty-ninth Annual Report of 1876, Farr pointed out that in the thirty years between 1847 and 1876, 106,565 mothers died in England in childbirth, or about 5 in every 1,000 or 1 to every 200 children born alive. The proportions varied from year to year, the lowest per 10,000 live born children being 42 in 1857 and the highest, 69 in 1874. In 1876 it was 47.

TABLE II.

Maternal death rates 10,000 live births from causes connected with the puerperal state, in England and Wales, 1847–1876, together with the percentage contributed by each separate cause to the whole number of such deaths.

(Condensed from the table of Dr. William Farr, Thirty-ninth Annual Report of the Registrar-General, 1876, page 279.)

1847–1876, inclusive.	Living births.	Accidents of child birth.			Metria.			Metria and child birth.	
		Deaths.	Rate per 10,000 births.	Per cent. total metria and child birth.	Deaths.	Rate per 10,000 births.	Per cent. total metria and child birth.	Deaths.	Rate per 10,000 Births
Total....	21,292,995	68,241	32.05	64.04	38,324	18.00	35.96	106,565	50.05

TABLE III.

Maternal death rates per 10,000 live births from causes connected with the puerperal state, and the percentage contributed by each separate cause to the whole number of such deaths, in England and Wales, 1911–1915.

Year.	Living births.	131			135			136			138			139			140			141			Total puerperal state, excluding puerperal septicemia.			137			Total puerperal state.	
		Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.						Puerperal septicemia.				
		Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1911	881,138	286	3.25	8.38	490	5.56	14.36	332	3.77	9.73	632	7.17	18.52	360	4.09	10.55	46	0.52	1.35	5	0.06	0.15	2,151	24.41	63.02	1,262	14.32	36.98	3,413	38.73
1912	872,737	329	3.77	9.47	522	5.98	15.03	378	4.33	10.88	629	7.21	18.11	362	4.15	10.42	30	0.34	0.86	7	0.08	0.20	2,257	25.86	64.99	1,216	13.93	35.01	3,473	39.79
1913	881,890	377	4.27	10.80	507	5.75	14.52	374	4.24	10.71	755	8.56	21.62	332	3.76	9.51	28	0.32	0.80	11	0.12	0.32	2,384	27.03	68.27	1,108	12.56	31.73	3,492	39.60
1914	879,096	403	4.58	10.99	465	5.29	12.68	396	4.50	10.80	659	7.50	17.97	332	3.78	9.05	40	0.46	1.09	7	0.08	0.19	2,302	26.19	62.78	1,365	15.53	37.22	3,667	41.71
1915	814,614	365	4.48	10.71	465	5.71	13.64	409	5.02	12.00	626	7.68	18.37	292	3.58	8.57	34	0.42	1.00	16	0.20	0.47	2,207	27.09	64.76	1,201	14.74	35.24	3,408	41.84
Total	4,329,475	1,760	4.07	10.08	2,449	5.66	14.03	1,889	4.36	10.82	3,301	7.62	18.91	1,678	3.88	9.61	178	0.41	1.02	46	0.11	0.26	11,301	26.10	64.75	6,152	14.21	35.25	17,453	40.31

Table II is condensed from Farr's table of the annual mortality rates for this thirty year period as given on page 249 of the Thirty-ninth Annual Report.

Farr blamed the high mortality of childbearing upon the poorly trained midwives, for in the experience of the Royal Maternity Charity, with two physicians and twenty-two trained and supervised midwives, of 9,019 deliveries of women in their homes during the three year period 1875 to 1877 there were lost only 21 women or 2.33 per 1,000 deliveries. The Birmingham Lying-in-Charity, in 8,607 deliveries, had a maternal mortality of 2.32 per 1,000. In 4,390 deliveries at Canterbury, Mr. Ridgen lost only 2.05 mothers per 1,000.

For comparison with the foregoing and with tables for the United States which follow, the subjoined table is presented, giving the mortality in childbirth per 10,000 living children, both total and under the rubrics 134–141, inclusive, of the international classification of the causes of death, for England and Wales for the five year period 1911 to 1915, as stated in the annual reports of the Registrar-General (see Table III).

The annual reports of the Registrar-General for England and Wales still carry rates of childbed mortality calculated on the basis of children reported born alive.

From the Swedish figures, notable for the care and accuracy with which they have been collected since 1746, Hendriks (1) has calculated rates showing the proportion of maternal deaths in childhood to women delivered, from 1776 to 1855, from which Table IV has been constructed.

TABLE IV.

Years.	Deaths to 100 deliveries.	Years.	Deaths to 100 deliveries.
1776–1780	0.89	1816–1820	0.73
1781–1785	0.83	1821–1825	0.67
1786–1790	0.93	1826–1830	0.66
1791–1795	1.01	1831–1835	0.57
1796–1800	0.85	1836–1840	0.51
1801–1805	0.78	1841–1845	0.44
1806–1810	0.89	1846–1850	0.44
1811–1815	0.79	1851–1855	0.44

It would appear by inference to be probable, though he does not so state, that in arriving at his figures for "women delivered," Hendriks made allowances for plural and for still births.

The writings of Mathews Duncan and of various other obstetrical writers on this subject may for the present purpose be dismissed as being based upon data of questionable accuracy, or of selected material or, of insufficient numbers. Some of the material is of value, however; as, for instance, that from certain lying-in charities and private practitioners, of which those quoted from Farr will serve as examples, for account is taken of the total number of deliveries and the maternal fatalities are presumably limited to those associated with labor.

The recent interesting article of Dublin (2), in which are compared the deaths from puerperal diseases occurring among a selected but large group of women insured in an industrial insurance company, with similar deaths among women in the registration area of the United States, has only an indirect bearing upon the questions under consideration, for the calculations are based upon the number of "females of the childbearing ages, namely, fifteen to forty-four."

However, none of the rates above considered are specific in the sense that they indicate with certainty the actual danger of childbearing, and to determine their real significance it is necessary to analyze the factors entering into both divisor and dividend. If the divisor embraced only the sum of the living and still births actually occurring, it would, when corrected for the number of plural births, represent the total number of pregnancies, which, developing to or near to the period of viability, eventuate in natural or artificially induced labor. Since the births of fetuses in the earlier months of pregnancy are not included, the total number of conceptions is not measured. Therefore, such figures are applicable not for determining the risk to life involved in becoming pregnant, but for ascertaining the risk run by those pregnant women who bear either live children or "stillborn" children. Hence, it is essential to establish just what the term *stillborn* defines.

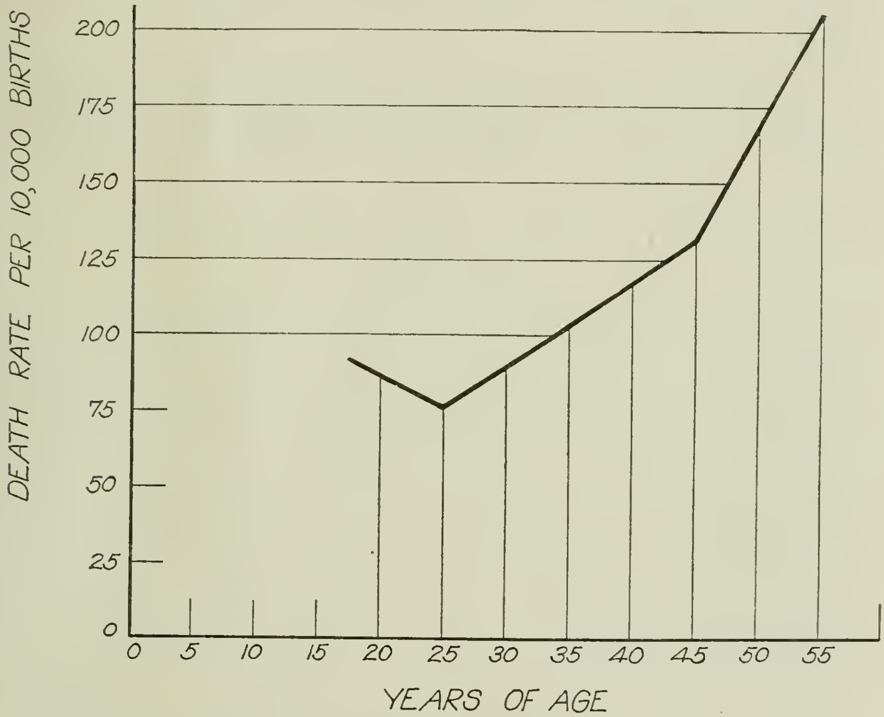
In the *Report of the Bureau of the Census on Birth Statistics* for the Birth Registration Area of the United States, for 1918, it is pointed out (pages 28 and 29) that the data relating to stillbirths must be interpreted with caution. Since the term *stillbirth* is not used in the same sense in the various states, the percentage of completeness of these reports is not known. The "model law" reads: "A stillborn child shall be registered as a birth and also as a death . . . provided that a certificate of birth and a certificate of death shall not be required for a child that has not advanced to the fifth month of uterogestation." Minnesota, New York, and North Carolina have similar provisions; the District of Columbia requires a certificate for a fetus "passed the fifth month"; the Indiana law reads "seven months gestation and over"; and the remaining states use the term *stillbirth* without defining it. Since Maryland makes especial efforts to secure uniform registration of stillbirths of even the earlier months of utero-gestation, the data for this state are not considered comparable with those of other states. It is held, however, that, since the figures of those states requiring reports only of children who have reached the fifth month approximate the figures of those states which do not define the term *stillbirth*, the conclusion is warranted that in the latter states only the stillbirths of the later months are for the most part reported; therefore, the figures are probably comparable. It is argued that the stillbirths of the later months of gestation must be well reported, because such stillbirths are interred, and burial cannot legally take place without a burial permit issued upon a physician's certificate. The data relating to stillbirths were compiled from transcripts of stillbirths reported as births, which has certainly resulted in figures smaller than would have been obtained had the transcripts of stillbirths reported as deaths been used.

Under the terms above outlined, stillbirths include some fetuses born dead before the period of viability. Since some physicians and midwives, in a small but not accurately known proportion of instances, do return as stillbirths the births of embryos of less than the fifth month of gestation, and others neglect to report the birth of some fetuses advanced beyond the fifth month and born dead, it is probably a fair assumption that these two factors balance each other, and consequently that the number of instances of stillbirths so reported represent a fair approximation of the actual number of fetuses of five months and over of utero-gestation born dead, in the birth registration area as a whole.

The number of living births reported does not, of course, in all, or perhaps in any, of the divisions of this registration area, represent the whole number of children born alive, for the reporting of live births is probably nowhere in the United States absolutely accurate. On the basis of the figures for plural and single births (both live and still) as given in the *Report of the Bureau of the Census on Birth Statistics* for 1918, the proportion of the former to the latter is as 1.15 to 100, or approximately 1 per cent.—the same percentage found by Farr for England and Wales, in 1852. It would, therefore, tend rather to decrease than to add to accuracy, to attempt to correct the figures for live births by counting the birth products of each plural pregnancy as a single unit. Attention must be called to two additional factors, the value of which is not in the present state of knowledge exactly ponderable—there must be greater risk involved in the parturient act for two or more children than for one, even should it be found that multiple pregnancy does not in itself involve greater danger to the mother than single pregnancy; and, no allowance is made for the difference in the danger between first and succeeding pregnancies. The birth rate, i.e., the rate of reported live births per 1,000 total living population, for the whole birth registration area was 24.4 in 1918, a figure, which, when compared with New York City (23.51 for 1918) where the birth registration is known to be exceptionally complete, would signify that the registration of births in the registration area is fairly accurate.

With these explanations and reservations, it may be concluded that the sum of the reported live and still births, as compiled by the Bureau of the Census, furnishes a fairly accurate, though not an absolutely correct, estimate of the total number of single labors occurring among women five months pregnant and over, in the birth registration area during 1918.

The dividend includes all the deaths classified under rubrics 134 to 141, inclusive, of the international classification of the causes of death, occurring in the cities and rural districts of the states of the birth registration area. Except where otherwise stated (in the case of certain cities in Tables VIII, IX, X, XI, XII, and XIII), the figures were obtained from the Mortality Statistics of the Bureau of the Census for 1918, which, in turn are compiled in the Bureau of the Census from transcripts of the original death certificates returned to local registrars. The classification of the deaths under the various rubrics may thus be considered as done with consistency for the whole registration area, after the certificates have been subjected to a double scrutiny to provide against error fraudulent or otherwise. Throughout this classification, as in that of the English tables, the deaths are classified specifically. Deaths of childbearing women due to other and ordinary causes are excluded; for example, deaths of childbearing women from influenza, phthisis, heart disease, cancer, etc., are classified under the appropriate primary cause—the childbearing state being reckoned a secondary cause. The influenza epidemic of 1918, therefore, had no appreciable influence upon the number of deaths classified as due to childbirth. As they stand, however, neither the total figures nor those under the separate rubrics can be accepted as representing accurately the deaths occurring in women bearing children of five months fetation and over, since these figures represent the recorded deaths of women dying in any part of the pregnant state from causes connected specifically with that condition. Under some of the eight rubrics, especially numbers 134, 136, 137, 138, and 139. there are probably recorded a not inconsiderable number of deaths of women before the fifth month of pregnancy. Accidents of pregnancy (134) includes accidental abortions, miscarriages, extra-uterine pregnancy, and vomiting of pregnancy; puerperal hemorrhage (135) may be fatal at any period of pregnancy. Some deaths due to deformities of mother or child, to injuries and operations (136), and to puerperal infection (137) undoubtedly occur before the fifth month of pregnancy, and the same may be said of the toxemias, including renal



Graph I illustrating death rate of mothers per 10,00 births, in reference to age.

affections and convulsions (138). Any of the various conditions enumerated under 139 may occur after abortions and miscarriages. The last two rubrics include but a small proportion of the total fatalities. Hence, the total number of deaths officially classified under the puerperal state (rubrics 134 to 139) represents not accurately, but only approximately, the number of deaths from these causes, of women five months pregnant and over.

Therefore, in both divisor and dividend, there are minor errors for which adequate allowances for correction cannot be made under the conditions by which the data are collected and recorded. It is equally clear, however, that the method here employed should measure much more accurately than any hitherto used the risk-rate to women five months pregnant and over of dying from causes connected with childbearing.

The field being cleared in the foregoing historical review and the detailed exposition of the factors involved presented, the application of the proposed method to the analysis of the data from various sources will be undertaken.

Risk-rate of childbearing in the birth registration area of the United States.

Since it furnishes a material larger in size and more homogeneous in character than any other available, the birth registration area of the United States for 1918 will be considered first. This area comprises the District of Columbia and the following states: Connecticut, Indiana, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, New Hampshire, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, Utah, Vermont, Virginia, Washington, and Wisconsin. It is estimated that this area contains 53 per cent. of the population of the United States. In it are situated besides the great cities of the Atlantic coast and of the Great Lakes region (excluding Chicago, however), the great number of smaller cities of New England, New York, Pennsylvania, Ohio, Kentucky, and of some of the western states. Classed as "cities" are those places of 10,000 inhabitants or more, according to the census of 1910. Since there were many changes in the eight years which elapsed, the distinction between urban and rural population is not so exact as would be desirable. The rural population, therefore, is by no means so truly rural—that is, village and farm dwelling—as this term is usually taken to imply. The frankly rural portion of the population dwells under very widely varying conditions of density, of climate, and of economic status, and, as is well known, it is certainly not homogeneous as to race stock. There is also, on the whole, but relatively little uniformity of the urban population in these and other important particulars. In regard to both urban and rural populations there is the greatest diversity in the character of obstetrical care. The total number of births, 1,412,283, for the area under consideration in 1918 is almost equally divided between urban and rural communities.

The figures for the maternal deaths are taken from the Mortality Statistics of the Bureau of the Census for 1918. To the possible criticism that during this year the number of maternal deaths was seriously influenced by the great epidemic of influenza, it may be said that, both in theory and in practice, this factor is eliminated in the classification of the deaths by the Bureau of the Census. The tables for New York, Philadelphia, and Baltimore to be presented later clearly indicate, so far as the official classification shows, that influenza did not affect the number of deaths from causes connected with childbirth. At any rate, choice here is limited by necessity.

The results of the analysis of the whole birth registration area are set forth in Table V. The death rates, whether viewed in comparison with the rates for England and Wales or considered alone, are appallingly high for total deaths and for deaths under each important category. These rates, although calculated by a method more favorable than that used in comparative tables in which stillbirths are not taken into account, are probably unparalleled in modern times in a civilized country. To match the rate for the whole puerperal state (88.48 per 10,000 births), it is necessary to revert to the Swedish rate for 1806–1810 (89 per 10,000 births). It is more than double the Swedish rate for 1851–1855, 75 per cent. greater than the average rate for England and Wales for 1847 to 1876 (50 per 10,000 living births), and 120 per cent. greater than the average rate for the latter area for 1911–1915 (40.31 per 10,000 living births), though the rate for the United States birth registration area—in which an allowance of 3.44 per cent. is made for stillbirths—is calculated on terms more favorable than that for England and Wales.

Considering first the rates for the United States birth registration area as a whole, it is evident that the risk to mothers in childbirth is excessive not only from all causes (88.48 per 10,000 deliveries, or 1 in 113), but from nearly every one of the separate causes. The rate for puerperal fever is 24.59 per 10,000 or 1 in every 407 deliveries, and for

TABLE V.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by the causes under each particular rubric to the whole number of such deaths, together with the percentage of still to total births, in the birth registration area of the United States for 1918.

1918	Births.				134			135			136			138			139			140			141			Total puerperal state, excluding puerperal septicemia.			137			Total puerperal state.	
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.						Puerperal septicemia.				
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
Urban.	710,886	686,561	24,325	3.42	1,542	21.69	23.40	437	6.15	6.63	1,126	15.84	17.10	1,290	18.15	19.58	158	2.22	2.40	35	0.49	0.53	8	0.11	0.12	4,596	64.65	69.75	1,993	28.04	30.25	6,589	92.69
Rural. .	701,397	677,088	24,309	3.47	1,492	21.27	25.26	417	5.95	7.06	1,133	16.15	19.18	1,206	17.19	20.42	149	2.12	2.52	27	0.38	0.46	3	0.04	0.05	4,427	63.12	74.94	1,480	21.10	25.06	5,907	84.22
Total. .	1,412,283	1,363,649	48,634	3.44	3,034	21.48	24.28	854	6.05	6.83	2,259	16.00	18.08	2,496	17.67	19.97	307	2.17	2.46	62	0.44	0.50	11	0.08	0.09	9,023	63.89	72.21	3,473	24.59	27.79	12,496	88.48

all other causes combined, 63.89 per 10,000, or 1 in every 156 deliveries. Of all the women delivered, there dies 1 in 565 of puerperal albuminuria and eclampsia, 1 in 411 of accidents of pregnancy, and 1 in 625 of accidents of labor. The various prominent causes stand in proportion of their relation to the whole number of childbed deaths as follows.

	Per Cent.
Puerperal fever	27.79
Accidents of pregnancy	24.28
Puerperal albuminuria and convulsions	19.97
Accidents of labor	18.08
Puerperal hemorrhage	6.85

The other causes are of comparatively slight importance.

To return to the comparison with England and Wales, in order to form a correct idea of the facts disclosed by this analysis, the rates in the birth registration area of the United States exceed the rates in England and Wales (see Table III) by nearly 75 per cent. for puerperal fever and 150 per cent. for the other affections of the puerperal state combined. In the important categories of the latter group, accidents of pregnancy and labor, the rates for the United States birth registration area as compared with the English rates are greater by over five times for the former and nearly four times for the latter. There is but little difference between the rates in the two countries for puerperal hemorrhage. Puerperal fever is responsible for 27.79 per cent. of the whole number of deaths in the puerperal state in the United States birth registration area and for 35.24 per cent. in the English, while all other causes in the puerperal state cover 72.21 per cent. in the former and 64.75 per cent. in the latter. (See Tables III and V.)

It will be noted that for the birth registration area of the United States, the total rate and the rate for puerperal septicemia are considerably lower in the rural than in the urban communities, while the rates for the other separate categories are approximately the same; therefore, it would appear that the more favorable rural total rate is due chiefly to the marked difference in the proportion of deaths from puerperal septicemia in rural as compared with urban districts. These urban rates for the birth registration area are considerably higher in each category than the corresponding rates for the large eastern medical centres, New York, Philadelphia, Boston, and Baltimore. (See Tables X, XI, XII, and XIII.) This difference is very

TABLE VI.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by the causes under each particular rubric to the whole number of such deaths, together with the percentage of still to total births, calculated for white and colored, urban and rural, in Kentucky, Maryland, North Carolina, and Virginia, for 1918.

	Births.				134			135			136			138			139			140			141			Total puerperal state, excluding puerperal septicemia.			137			Total Puerperal state .	
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.						Puerperal septicemia				
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
Urban White	33,512	32,749	763	2.28	92	27.45	25.34	15	4.48	4.13	53	15.82	14.60	93	27.75	25.62	6	1.79	1.65	2	0.60	0.55	—	—	—	261	77.88	71.90	102	30.44	28.10	363	108.32
Rural white	154,669	149,364	5,305	3.43	338	21.85	27.61	84	5.43	6.86	244	15.78	19.93	270	17.46	22.06	11	0.71	0.90	6	0.39	0.49	2	0.13	0.16	955	61.74	78.02	269	17.39	21.98	1,224	79.14
Urban colored	9,006	8,360	646	7.17	16	17.77	9.76	6	6.66	3.66	21	23.32	12.80	54	59.96	32.93	7	7.77	4.27	—	—	—	1	1.11	0.61	105	116.59	64.02	59	65.51	35.98	164	182.10
Rural colored	47,811	44,565	3,246	6.79	100	20.92	17.76	30	6.27	5.33	107	22.38	19.01	179	37.44	31.79	6	1.25	1.07	—	—	—	—	—	—	422	88.26	74.96	141	29.49	25.04	563	117.76
Total white	188,181	182,113	6,068	3.22	430	22.85	27.10	99	5.26	6.24	297	15.78	18.71	363	19.29	22.87	17	0.90	1.07	8	0.43	0.50	2	0.11	0.13	1,216	64.62	76.62	371	19.72	23.38	1,587	84.33
Total colored	56,817	52,925	3,892	6.85	116	20.42	15.96	36	6.34	4.95	128	22.53	17.61	233	41.01	32.05	13	2.29	1.79	—	—	—	1	0.18	0.14	527	92.75	72.49	200	35.20	27.51	727	127.95
Total urban	42,518	41,109	1,409	3.31	108	25.40	20.49	21	4.94	3.98	74	17.40	14.04	147	34.57	27.89	13	3.06	2.47	2	0.47	0.38	1	0.24	0.19	366	86.08	69.45	161	37.87	30.55	527	123.95
Total rural	202,480	193,929	8,551	4.22	438	21.63	24.51	114	5.63	6.38	351	17.34	19.64	449	22.18	25.13	17	0.84	0.95	6	0.30	0.34	2	0.10	0.11	1,377	68.01	77.06	410	20.25	22.94	1,787	88.26
Total....	244,998	235,038	9,960	4.07	546	22.29	23.60	135	5.51	5.83	425	17.35	18.37	596	24.33	25.76	30	1.22	1.30	8	0.33	0.35	3	0.12	0.13	1,743	71.14	75.32	571	23.31	24.68	2,314	94.45

likely due in part to poorer obstetrical care in the large cities of the interior and in the smaller cities throughout the territory included in the registration area.

Since the figures for the still and live births and for the maternal deaths in the puerperal state are tabulated for white and colored in the registration states of Kentucky, Maryland, North Carolina, and Virginia, it is possible to determine the difference in the force of mortality in childbirth in white and colored women under both urban and rural conditions. The results of the calculations are shown in Table VI. Because there are but few Mongolian and Indian females in the populations of these states, the term *colored* may for all practical purposes be taken to express women of negro or partly negro stock. Since the chief purpose of the comparison is to investigate influences of race stock upon childbirth mortality, *negro* is used in these remarks instead of the official word *colored*. Though in the area now under consideration the urban population numbers somewhat less than one fifth of the rural, nevertheless the sample of the former is of considerable size and includes Baltimore, Richmond, and Louisville, comparatively large cities with conspicuously large negro populations. In the four states, the total rate and the urban and rural rates for all causes connected with the puerperal state are considerably in excess of those of the whole registration area. The rates for total deaths stand in order of their favorableness: white rural, total white, white urban, colored rural, total colored, colored urban. For puerperal septicemia, the total and the total rural and the rural white and the total white compare favorably with those for the registration area as a whole (23.31, 20.25, 17.39, and 19.72, respectively, to 24.59). The differences in the ratio for this cause between urban and rural rates for total, white, and colored, and particularly for the latter, is marked. The total colored is nearly double that of the total white. For the total puerperal state, puerperal fever excluded, the total rate and the urban and rural rates all are higher than those for the whole registration area. The urban rate is always higher than the rural, and the colored rate, than the white. While the accidents of pregnancy show a higher rate in the white than in the negro, the reverse is true for accidents of labor—a set of causes more particularly associated with deformities and with delivery. Puerperal hemorrhage is in none of the groups a conspicuous cause of death, the average being 5.83 per cent. of the total deaths. It is less fatal in the city dwellers than in the rural dwellers of both white and negro races, and in the white, less fatal for the former than for the latter community. Other accidents of labor, causing over 18 per cent. of the total deaths, has approximately identical rates for city and country in both races, but is greater by a third for negroes than for whites. Puerperal albuminuria and convulsions, responsible for 25.76 per cent. of the deaths and markedly higher in the urban than in the rural population for both races, is twice as fatal for the negro as for the white woman. The other two categories of causes play parts too minor to make them of serious account.

In these four states, in which the Anglo-Saxon stock greatly preponderates among those of white blood, and notwithstanding the relatively favorable influence exerted by the lower rate among the whites in Baltimore (62.02 per 10,000 deliveries), the death rate in childbearing (84.33 per 10,000), though somewhat lower than in the registration area as a whole (88.48 per 10,000), is markedly higher than any recorded for England and Wales since 1842 and nearly twice that of Sweden in 1855. The total rate for negroes (127.95 per 10,000) is considerably higher than the same rate in Baltimore (109.54 per 10,000), and the urban rate (182.10 per 10,000) is 55 per cent. greater than the rural rate (117.76 per 10,000.) The urban rate is nearly 2 per 100, or about 1 in 45 deliveries, while in the country 1 out of about every 58 childbearings is fatal to the mother. Since the proportion of stillborn is so high among the negroes, the sacrifice of mothers in producing live children is even greater than these figures indicate. Though further and more intensive studies based upon accurate and complete histories of a sufficient number of cases are necessary to determine definitely the reasons for the greatly higher maternal mortality in the negro over the white in childbearing, the evidence at hand suggests that the causes at work are concerned in considerable degree, at least, with difference in degree of racial susceptibility to puerperal infection, albuminuria and convulsions (the toxemias of pregnancy and eclampsia), and to accidents of labor. The rates of Table VI show that, whether in city or in country, the mortality from these causes is much in excess among the negroes.

Influence of age upon risk-rate of childbearing in the birth registration area of the United States.

In Table VII, the births and the maternal deaths by age periods and the rates calculable therefrom are given for the birth registration

TABLE VII.

Maternal death rates per 10,000 births (live and still) according to age of mother, from causes connected with the puerperal state, and the percentage contributed by the causes under each particular rubric to the whole number of such deaths, together with the percentage of still to total births, for the birth registration area of the United States, exclusive of Maine, Massachusetts, Rhode Island, and Washington, for 1918.

(Data on stillbirths for Baltimore, Maryland, obtained from the Baltimore Health Department.)

Age period.	Births.				134 Accidents of pregnancy.			135 Puerperal hemorrhage.			136 Other accidents of labor.			138 Puerperal albuminuria and convulsions.			139 Puerperal phlegmasia, alba dolens, embolus, sudden death.			140 Following childbirth, not otherwise defined.			141 Puerperal diseases of the breast.			Total puerperal state, excluding Puerperal septicemia.			137 Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
10–19....	102,768	98,232	4,536	4.41	183	17.81	19.26	22	2.14	2.32	132	12.84	13.89	317	30.85	33.37	8	0.78	0.84	3	0.29	0.32	1	0.10	0.11	666	64.81	70.11	284	27.64	29.89	950	92.44
20–29....	705,563	681,926	23,637	3.35	1,488	21.09	27.68	244	3.46	4.54	1,009	14.30	18.77	1,007	14.27	18.73	112	1.59	2.08	28	0.40	0.52	6	0.09	0.11	3,894	55.19	72.43	1,482	21.00	27.57	5,376	76.19
30–39....	379,295	363,575	15,720	4.14	899	23.70	23.00	373	9.83	9.54	684	18.03	17.50	704	18.56	18.01	103	2.72	2.64	17	0.45	0.44	2	0.05	0.05	2,782	73.35	71.19	1,126	29.69	28.81	3,908	103.03
40–49....	55,807	52,468	3,339	5.98	99	17.74	13.52	116	20.79	15.85	120	21.50	16.39	167	29.92	22.81	23	4.12	3.14	—	—	—	1	0.18	0.14	526	94.25	71.86	206	36.91	28.14	732	131.17
50 and over...	243	219	24	9.88	1	41.15	20.00	—	—	—	1	41.15	20.00	1	41.15	20.00	—	—	—	—	—	—	—	—	—	3	123.46	60.00	2	82.30	40.00	5	205.76
Unknown	9,974	5,448	1,927	19.32	5	5.01	25.00	3	3.01	15.00	3	3.01	15.00	4	4.01	20.00	—	—	—	—	—	—	—	—	—	15	15.04	75.00	5	5.01	25.00	20	20.05
Total .	1,251,723	1,201,868	49,855	3.98	2,675	21.37	24.34	758	6.06	6.90	1,949	15.57	17.73	2,200	17.58	20.02	246	1.97	2.24	48	0.38	0.44	10	0.08	0.09	7,886	63.00	71.75	3,105	24.81	28.25	10,991	87.81

area as of 1918, with the omission of certain states—Maine, Massachusetts, Rhode Island, and Washington, for which comparative data could not be obtained. On account of the manner of age classification of deaths in the Mortality Reports of the Bureau of the Census, it is not possible to begin in the ten year periods with the fifteenth year as in Farr's Table.

It will be noted that for the first age group, 10 to 19 years, in which about one twelfth of the births occur, the total rate is higher than for the second ten year period, 20 to 29 years, but lower than for any other of the age divisions. The maternal death rates in this group, in which most of the labors probably occur in primiparae, are, in comparison with the second and third age groups, relatively low for all the important categories embraced under "total puerperal state, excluding puerperal fever," with the exception of the one category—albuminuria and convulsions. The rate for puerperal fever and the total rate are higher, and the rates for accidents of pregnancy and labor and for hemorrhage are lower than for the averages of all ages.

The second age group, in which over half of the labors fall, shows the lowest rates for total and for the important rubrics of septicemia and albuminuria and convulsions, and, in contrast to the succeeding age groups, in the rate for accidents of labor; for this category, as with all the other important ones, it is below that of the average for all ages. So far as can be determined by this method of age classification, this is, on the whole, the most favorbale age for childbearing.

The third and fourth age groups, 30 to 39 years and 40 to 49 years, including over a third of the labors, show in most of the categories progressively higher rates—the increase of the risk of death from hemorrhage, albuminuria and convulsions, and puerperal fever being conspicuous at these higher ages. The total rates are in consequence higher than at other ages.

The age group "over 50 years," with high rates in all the important categories, particularly in septicemia, has a total rate of 206.68 per 10,000. The sample, however, is too small to warrant fast conclusions.

The relative importance in the various age groups of the different categories is shown in the columns of percentages. The value of these from the practical standpoint of prognosis would be considerably enhanced, if it were possible to make the age groupings by quinquennial periods. The influence of the age of the mother upon the death rates from all causes connected with the puerperal state is indicated in Graph I (p. 207).

The risk-rate of childbearing in certain cities of Europe and the United States.

In the annual reports of European cities, which so far I have been able to obtain, there are only two, Stockholm, Sweden, and Birmingham, England, in which the stillbirths are reported and the maternal deaths are given under the separate rubrics over a series of years. For comparison with similar data for the below mentioned cities of the United States, I have compiled tables for these cities, showing the total rates and the rates for the different categories under deaths in the puerperal state. The data for these cities and for New York, Philadelphia, Boston, and Baltimore were obtained from the annual official health reports. Therefore, since the deaths were not classified in the Bureau of the Census, there may be some difference in their assignment in some of the categories, due to variations in custom or of opinion in the several statistical bureaus.

It will be observed that the classification used in Stockholm is somewhat different from that of the international classification and with fewer rubrics. Apparently, however, the latter include all the deaths occurring in the puerperal state (see Table VIII). Had the table for Stockholm been made to include the years 1910 to 1914, the showing for the city would have been even more favorable. Indeed, when the steady upward progress of the rates for puerperal septicemia since 1914, causing a doubling of the rate for that category and a large increase in the total rate, is taken into consideration, it would appear that during the World War factors favorable for this affection have been active in Stockholm, temporarily affecting the rates to a disadvantage. Hemorrhage, except from placenta previa, is probably included under "other accidents of labor." The rates for the total puerperal state are remarkably low. The unusually excellent results in the management of labor in Stockholm are probably explained partly by the physique of the women and partly by good obstetrical practice and the large extent to which hospitalization is exercised. The low percentage of stillbirths to total births supports this view.

The death rates for the puerperal state for Birmingham are probably among the lowest reached by any large city and are the result of efforts intelligently directed over a long series of years (see

TABLE VIII.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Stockholm, Sweden, 1915–1919

(Compiled from the annual reports of the Health Department of Stockholm.)

Year.	Births.				Abortion, difficult labor.			Placenta previa.			Convulsions, eclampsia.			All other accidents of labor.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total Puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1915...	7,082	6,913	169	2.39	4	5.65	20.00	–	—	—	1	1.41	5.00	2	2.82	10.00	7	9.88	35.00	13	18.36	65.00	20	28.24
1916...	6,736	6,579	157	2.33	2	2.97	8.33	–	—	—	3	4.45	12.50	4	5.94	16.67	9	13.36	37.50	15	22.27	62.50	24	35.63
1917...	6,758	6,634	124	1.83	–	—	—	–	—	—	2	2.96	9.52	1	1.48	4.76	3	4.44	14.29	18	26.64	85.71	21	31.07
1918...	6,026	5,887	139	2.31	2	3.32	5.71	2	3.32	5.71	3	4.98	8.57	2	3.32	5.71	9	14.94	25.71	26	43.15	74.29	35	58.08
1919...	5,560	5,454	106	1.91	–	—	—	–	—	—	2	3.60	6.67	3	5.40	10.00	5	8.99	16.67	25	44.96	83.33	30	53.96
Total..	32,162	31,467	695	2.16	8	2.49	6.15	2	0.62	1.54	11	3.42	8.46	12	3.73	9.23	33	10.26	25.38	97	30.16	74.62	130	40.42

TABLE IX.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by the causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Birmingham, England, for 1913, 1914, 1915, 1916, and 1918.

(Compiled from the annual reports of the Health Department of Birmingham.)

Years.	Births.				134 Accidents of pregnancy.			135 Puerperal hemorrhage.			136 Other accidents of labor.			138 Puerperal albuminuria and convulsions.			139 Puerperal phlegmasia, alba dolens, embolus, sudden death.			140 Following childbirth, not otherwise defined.			141 Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			137 Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1913...	24,491	23,812	679	2.77	8	3.27	8.69	14	5.72	15.22	10	4.08	10.87	9	3.67	9.78	7	2.86	7.61	—	—	—	—	—	—	48	19.60	52.17	44	17.97	47.83	92	37.56
1914...	22,600	21,838	762	3.37	9	3.98	12.16	10	4.42	13.51	11	4.87	14.86	9	3.98	12.16	2	0.88	2.70	—	—	—	—	—	—	41	18.14	55.41	33	14.60	44.59	74	32.74
1915...	21,919	21,187	732	3.34	6	2.74	8.22	10	4.56	13.70	8	3.65	10.96	10	4.56	13.70	3	1.37	4.11	—	—	—	1	0.46	1.37	38	17.34	52.05	35	15.97	47.95	73	33.30
1916...	21,347	20,618	729	3.41	2	0.94	2.82	10	4.68	14.08	5	2.34	7.04	13	6.09	18.31	9	4.22	12.68	1	0.47	1.41	—	—	—	40	18.74	56.34	31	14.52	43.66	71	33.26
1918...	17,430	16,840	590	3.38	4	2.29	7.84	8	4.59	15.69	3	1.72	5.88	4	2.29	7.84	3	1.72	5.88	—	—	—	—	—	—	22	12.62	43.14	29	16.64	56.86	51	29.26
Total..	107,787	104,295	3,492	3.24	29	2.69	8.03	52	4.82	14.40	37	3.43	10.25	45	4.17	12.47	24	2.23	6.65	1	0.09	0.28	1	0.09	0.28	189	17.53	52.35	172	15.96	47.65	361	33.49

Table IX.) It will be noted that the total rate has consistently fallen through the five-year period, three and one half years of which were during the World War. The rate for each of the important categories is strikingly low. It is evident, when comparison is made with the rates of New York, Philadelphia, Boston, and Baltimore, and with those of the birth registration area of the United States, that the low rates of this manufacturing city must, in large degree, be due to the character of obstetrical care afforded. These rates are not only so low, but relatively so well balanced, that they will be taken as standards of comparison for the American cities now to be discussed.

The average total rate for New York City (46.11 per 10,000 labors), conspicuously lower than that for any of the other cities of the United States under consideration, is over 35 per cent. higher than that for Birmingham (see Table X). The average rate for puerperal septicemia is not only lower than that for Birmingham, but the rate for this affection has fallen continuously throughout the five year period, and in 1919 reached the remarkably low figure of 11.41 per 10,000 labors. The reports of the Department of Health indicate that this result has been materially influenced by persistent and intelligently directed administrative measures. While the rates for the other categories are not so low as those for Birmingham, they are well below those for the other American cities in this group.

In Philadelphia, both the total rate (61.48 per 10,000 labors) and the rates for the important categories are well above those for New York—notably so for puerperal fever, the average rate (27.10 per 10.000 labors) being double that (14.41 per 10,000 labors) for the latter city (see Table XI). In accidents of labor, however, the record of Philadelphia is lower by half, but this is evidently due to classifying deaths under 140 which in New York would be assigned to 136.

The rates for Boston, for 1914 to 1918, are given in Table XII. The average rate for all causes connected with the puerperal state (70.71 per 10,000, or 1 in 141 births) is excessively high as compared with the other cities. The average rates for all the important rubrics are considerably higher than in New York, and with the exception of puerperal septicemia they are greater than those in Philadelphia. In regard to puerperal septicemia, Boston surpasses Baltimore, in spite of the large negro population of the latter city.

The average total rate for the puerperal state in Baltimore is not only very high (64.89 per 10,000, or about 1 in every 150 deliveries),

TABLE X.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for New York City, 1915–1919.

(Compiled from the annual reports of the Health Department of New York.)

Year.	Births.				134			135			136			138			139			140			141						137				
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and Convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1915. .	147,669	141,256	6,413	4.34	118	7.99	16.62	63	4.27	8.87	91	6.16	12.82	144	9.75	20.28	38	2.57	5.35	3	0.20	0.42	4	0.27	0.56	461	31.22	64.93	249	16.86	35.07	710	48.08
1916. .	143,917	137,664	6,253	4.34	78	5.42	11.94	85	5.91	13.02	69	4.79	10.57	158	10.98	24.20	40	2.78	6.13	1	0.07	0.15	1	0.07	0.15	432	30.02	66.16	221	15.36	33.84	653	45.37
1917. .	147,681	141,564	6,117	4.14	77	5.21	11.83	80	5.42	12.29	74	5.01	11.37	169	11.44	25.96	30	2.03	4.61	2	0.14	0.31	6	0.41	0.92	438	29.66	67.28	213	14.42	32.72	651	44.08
1918. .	144,839	138,046	6,793	4.69	95	6.56	14.31	83	5.73	12.50	61	4.21	9.19	184	12.70	27.71	24	1.66	3.61	10	0.69	1.51	5	0.35	0.75	462	31.90	69.58	202	13.95	30.42	664	45.84
1919. .	136,361	130,377	5,984	4.39	128	9.39	19.88	61	4.47	9.47	84	6.16	13.04	171	12.54	26.55	43	3.15	6.68	2	0.15	0.31	2	0.15	0.31	491	36.01	76.24	153	11.22	23.76	644	47.23
Total.	720,467	688,907	31,560	4.38	496	6.88	14.93	372	5.16	11.20	379	5.26	11.41	826	11.46	24.86	175	2.43	5.27	18	0.25	0.54	18	0.25	0.54	2,284	31.70	68.75	1,038	14.41	31.25	3,322	46.11

TABLE XI.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Philadelphia, 1915–1919.

(Compiled from the annual reports of the Health Department of Philadelphia.)

Year.	Births.				134			135			136			138			139			140			141						137				
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1915...	42,820	40,849	1,971	4.60	20	4.67	7.09	26	6.07	9.22	10	2.34	3.55	79	18.45	28.01	12	2.80	4.26	12	2.80	4.26	—	—	—	159	37.13	56.38	123	28.73	43.62	282	65.86
1916...	42,563	41,220	1,343	3.16	24	5.64	8.30	32	7.52	11.07	15	3.52	5.19	54	12.69	18.69	12	2.82	4.15	18	4.23	6.23	—	—	—	155	36.42	53.63	134	31.48	46.37	289	67.90
1917...	44,897	42,917	1,980	4.41	13	2.90	5.02	23	5.12	8.88	8	1.78	3.09	68	15.15	26.25	9	2.00	3.47	17	3.79	6.56	—	—	—	138	30.74	53.28	121	26.95	46.72	259	57.69
1918...	45,153	42,904	2,249	4.98	15	3.32	5.88	23	5.09	9.02	12	2.66	4.71	60	13.29	23.53	4	0.89	1.57	27	5.98	10.59	1	0.22	0.39	142	31.45	55.69	113	25.03	44.31	255	56.47
1919...	44,149	42,047	2,102	4.76	19	4.30	7.17	26	5.89	9.81	13	2.94	4.91	69	15.63	26.04	11	2.49	4.15	23	5.21	8.68	—	—	—	161	36.47	60.75	104	23.56	39.25	265	60.02
Total..	219,582	209,937	9,645	4.39	91	4.14	6.74	130	5.92	9.63	58	2.64	4.30	330	15.03	24.44	48	2.19	3.56	97	4.42	7.19	1	0.05	0.07	755	34.38	55.93	595	27.10	44.07	1,350	61.48

TABLE XII.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Boston, 1914 to 1918.

(Compiled from the annual report of the Health Department of Boston.)

Year.	Births.				134 Accidents of pregnancy.			135 Puerperal hemorrhage.			136 Other accidents of labor.			138 Puerperal albuminuria and convulsions.			139 Puerperal phlegmasia, alba dolens, embolus, sudden death.			140 Following childbirth, not otherwise defined.			141 Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			137 Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1914..	20,251	19,462	789	3.90	8	3.95	6.30	18	8.89	14.17	21	10.37	16.54	34	16.79	26.77	1	0.49	0.79	1	0.49	0.79	—	—	—	83	40.99	65.35	44	21.73	34.65	127	62.71
1915..	20,416	19,725	691	3.38	10	4.90	6.49	17	8.33	11.04	18	8.82	11.69	51	24.98	33.12	9	4.41	5.84	—	—	—	1	0.49	0.65	106	51.92	68.83	48	23.51	31.17	154	75.43
1916..	20,308	19,594	714	3.52	11	5.42	8.46	12	5.91	9.23	13	6.40	10.00	25	12.31	19.23	10	4.92	7.69	2	0.98	1.54	—	—	—	73	35.95	56.15	57	28.07	43.85	130	64.01
1917..	20,523	19,856	667	3.25	26	12.67	14.94	11	5.36	6.32	24	11.69	13.79	41	19.98	23.56	12	5.85	6.90	2	0.97	1.15	1	0.49	0.57	117	57.01	67.24	57	27.77	32.76	174	84.78
1918..	20,890	20,063	827	3.96	21	10.05	15.11	13	6.22	9.35	23	11.01	16.55	33	15.80	23.74	8	3.83	5.76	1	0.48	0.72	—	—	—	99	47.39	71.22	40	19.15	28.78	139	66.54
Total.	102,388	98,700	3,688	3.60	76	7.42	10.50	71	6.93	9.81	99	9.68	13.67	184	17.97	25.41	40	3.91	5.52	6	0.59	0.83	2	0.20	0.28	478	46.69	66.02	246	24.03	33.98	724	70.71

but unlike those of New York and Philadelphia shows an almost unbroken ascent and in 1919 is almost 40 per cent. higher than in 1915. This increase is in nearly every rubric, including septicemia. The average rate for the latter affection, however, is conspicuously lower than that for Philadelphia and for the birth registration area, but much higher than that for New York (see Table XIII). On the whole, the record for Baltimore, like that for Boston, is without a redeeming feature, even the rate for puerperal hemorrhage being about 40 per cent. above the average for that category in the other tables. On account of the large size of the negro population, Baltimore offers a good opportunity to compare the childbed death rates among negroes and whites in a large city. Negroes comprise about 2 per cent. of the populations in New York and Boston, and about 5 per cent. in Philadelphia. In Baltimore, about 15 per cent. or one sixth of both population and births, is negro. The two years, 1916 and 1919, for which data for comparison are obtainable, are by chance the years showing the lowest and the highest rates. It will be noted that the average of the total negro rate for these two years is almost double that of the white (109.54 to 62.02), the cause of the excess being the higher negro rates for accidents of labor, puerperal convulsions and albuminuria, and septicemia, all of which are extraordinarily high and double those of the whites (1 in 90 as against 1 in 160 deliveries). The rate for puerperal septicemia (40.44 per 10,000 births) in the negroes in Baltimore is higher than the total rate for the whole puerperal state in Birmingham and Stockholm, and not far from that in New York. It is interesting that the comparatively high rates for accidents of pregnancy and for puerperal hemorrhage in Baltimore are approximately the same for the two races.

According to information furnished me in 1917, about one half of the lying-in women receiving obstetrical care in the well organized in- and out-patient departments of the obstetrical clinics of the Johns Hopkins Hospital and of the University of Maryland Hospital are negroes, representing about 50 per cent. of the annual number of negro births in the city. The other half of the deliveries in these obstetrical services includes less than 10 per cent. of the white births. The deliveries in these institutions represent approximately 15 per cent. of the annual births in the City, so about 50 per cent. of the negro and about 10 per cent. of the white labors occur under these auspices. Notwithstanding these relatively very favorable opportuni-

TABLE XIII.

Maternal death rate per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Baltimore. Corresponding rates and percentages are calculated for white and colored for 1916 and 1919.

(Compiled from the annual reports of the Health Department of Baltimore.)

Year.	Births.				134			135			136			138			139			140			141						137				
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1915..........	14,765	13,634	1,131	7.66	11	7.45	13.75	10	6.77	12.50	6	4.06	7.50	22	14.90	27.50	6	4.06	7.50	—	—	—	—	—	—	55	37.25	68.75	25	16.93	31.25	80	54.18
1916..........	16,320	15,085	1,235	7.57	17	10.42	16.35	12	7.35	11.54	8	4.90	7.69	28	17.16	26.92	4	2.45	3.85	—	—	—	—	—	—	69	42.28	66.35	35	21.45	33.65	104	63.73
1917..........	16,217	14,950	1,267	7.81	8	4.93	8.79	11	6.78	12.09	7	4.32	7.69	31	19.12	34.07	2	1.23	2.20	—	—	—	—	—	—	59	36.38	64.84	32	19.73	35.16	91	56.11
1918..........	16,492	15,292	1,200	7.28	30	18.19	25.21	12	7.28	10.08	14	8.49	11.76	31	18.80	26.05	1	0.61	0.84	—	—	—	—	—	—	88	53.36	73.95	31	18.80	26.05	119	72.16
1919..........	18,958	17,631	1,327	7.00	21	11.08	14.69	19	10.02	13.29	21	11.08	14.69	38	20.04	26.57	4	2.11	2.80	—	—	—	—	—	—	103	54.33	72.03	40	21.10	27.97	143	75.43
Total.........	82,752	76,592	6,160	7.44	87	10.51	16.20	64	7.73	11.92	56	6.77	10.43	150	18.13	27.93	17	2.05	3.17	—	—	—	—	—	—	374	45.20	69.65	163	19.70	30.35	537	64.89
1916 white .	13,503	12,662	841	6.23	14	10.37	18.92	11	8.15	14.86	5	3.70	6.76	18	13.33	24.32	3	2.22	4.05	—	—	—	—	—	—	51	37.77	68.92	23	17.03	31.08	74	54.80
colored	2,817	2,423	394	3.99	3	10.65	10.00	1	3.55	3.33	3	10.65	10.00	10	35.50	33.33	1	3.55	3.33	—	—	—	—	—	—	18	63.90	60.00	12	42.60	40.00	30	106.50
1919 white .	15,841	14,908	933	5.89	18	11.36	16.67	15	9.47	13.89	16	10.10	14.81	27	17.04	25.00	4	2.53	3.70	—	—	—	—	—	—	80	50.50	74.07	28	17.68	25.93	108	68.18
colored	3,117	2,723	394	12.64	3	9.62	8.57	4	12.83	11.43	5	16.04	14.29	11	35.29	31.43	—	—	—	—	—	—	—	—	—	23	73.79	65.71	12	38.50	34.29	35	112.29
Total white..	29,344	27,570	1,774	6.05	32	10.91	17.58	26	8.86	14.29	21	7.16	11.54	45	15.34	24.73	7	2.39	3.85	—	—	—	—	—	—	131	44.64	71.98	51	17.38	28.02	182	62.02
Total colored	5,934	5,146	788	13.28	6	10.11	9.23	5	8.43	7.69	8	13.48	12.31	21	35.39	32.31	1	1.69	1.54	—	—	—	—	—	—	41	69.09	63.08	24	40.44	36.92	65	109.54

* In number of stillborn white are included, 3 color unknown and 2 sex and color unknown.

ties for lying-in care, the Baltimore negress is a very poor maternity risk. Her chances are materially better, however, than in the four states analyzed in Table VI.

In this connection, comparison may be made with Washington, D. C., rates for which I have calculated for 1918 on the basis of figures from the census reports. With 8,673 births (living and still), 6,298 white and 2,375 colored, and 74 maternal deaths, 47 white and 27 colored, the total maternal death rate was 85.32 per 10,000 births —74.63 for white and 113.68 for colored. Thus, in the National Capital, the risk rate for childbirth for the negro exceeded that in Baltimore.

STILLBIRTHS.

The question of the relative frequency of stillbirths as compared with live births is one of great interest and importance. That the proportion varies in the same country at different times and places and in different countries is to be expected. Owing to uncertainties as to what the term *stillbirth* is used to comprehend at different times and in various localities, absolute comparisons are unwarranted and minute corrections are impossible.

For the sake of comparison, figures for Sweden and other continental countries taken from the tables of Hendriks (loc. cit., p. 167) and figures from my tables V to XIII are given in Table XIV. In regard to Sweden, Hendriks showed that from 1776 to 1855, on an average to every 100 deliveries there were born 101.62 live and still born children, of which 98.80 were live and 2.80 still born; during the five year period, 1851 to 1855, these proportions were in the rural districts 101.39, 98.27, 3.12, and in towns, 101.46, 96.98, and 4 48.

It will be noted that the percentage of stillbirths for Sweden was much lower than in any of the other countries given in Hendriks table and that the percentage in Stockholm is at the present time considerably below that of any of the other cities in my table. In comparison with the experience of Swedish towns in 1851 to 1855, the stillbirth percentage of this city is now lower by one half. This relatively low percentage of stillbirths in Sweden and its chief city is probably influenced by the same factors already suggested as explaining the low death rates in maternity; i.e., the physique of the women and the superior obstetrical care. However, that low maternal death rates and low percentages of stillbirths do not of necessity go together is evident from the figures of Birmingham, New York, and Boston. The former city with its remarkably low maternity death rates has a stillbirth rate nearly as high as that of Boston, where the maternity death rates are notably high. Philadelphia, with a maternity death rate greater by more than 30 per cent. than that of New York, has the same stillbirth rate. In the birth registration area as a whole and in the four states, Kentucky, Maryland, North Carolina, and Virginia, taken together, with maternity death rates much higher than that for New York, the stillbirth rates, whether total, urban or rural are considerably lower than in that city. The stillbirth rate for the whole birth registration area of the United States is but very little higher than that of Birmingham, whose maternal death rate is, on the other hand, less by considerably more than 60 per cent.* The very high stillbirth rate of Baltimore,

TABLE IV.

Hendriks.			Howard.		
City or country.	Date.	Average percentage of still to total births.	City or country	Date.	Average percentage of still to total births.
Sweden	1776–1855	2.77	Stockholm	1915–1919	2.16
" Rural	1851–1855	3.12	Birmingham	1913–1918	3.24
" Urban	1851–1855	4.48	New York	1915–1919	4.38
Norway	1836–1845	4.08	Philadelphia	1915–1919	4.39
Denmark	1845–1849	4.26	Boston	1917–1918	3.60
Hannover	1853	3.87	Baltimore	1915–1919	7.44
Prussia	1825–1849	3.69	" White	"	6.05
Saxony	1847–1849	4.49	" Negro	"	13.28
Bavaria	1844–1851	3.3	U. S. Birth Registration Area	1918	3.44
Netherlands	1848–1853	4.97	U. S. Birth Registration Area, Urban	"	3.42
Belgium	1841–1850	4.18	U. S. Birth Registration Area, Rural	"	3.47
France	1851–1854	3.79	Kentucky, Maryland, North Carolina, and Virginia	"	4.07
			Total Urban	"	3.31
			Total Rural	"	4.22
			Total White	"	3.22
			Urban White	"	2.28
			Rural White	"	3.43
			Total Negro	"	6.85
			Urban Negro	"	7.17
			Rural Negro	"	6.79

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* The explanation of this seeming paradox must be sought for in the operation of some potent cause of maternal death, which is at the same time without

in both white and negro is due to some degree to the custom of reporting as stillbirths fetuses of earlier months of utero-gestation, partly under the influence of the state authorities and partly because of the response of physicians to requests for stillborn fetuses for scientific investigation in the laboratories of embryology. It is probably not far from the truth to assume that on the same basis of birth reporting in other places, the percentage of still to total births in Baltimore is not far from 5 in whites and 11 in negroes—rates of fetal fatality of considerable eminence. The percentage of stillbirths to total births in Washington, D. C., for 1918, was 5.89 for the whole number, 4.40 for white, and 9.85 for negro. It is significant that here as in the four states, Kentucky, Maryland, North Carolina, and Virginia, the stillbirth rate for negroes is double that for whites. In this division of the registration area, the stillbirth rate for whites is lower in the cities than in the rural districts, while for negroes, the opposite is the case.

It has long been known that fetal fatality is influenced conspicuously by causes inherent in the fetus itself (namely, errors of implantation and of development) and by accidents *in utero*—malposition of fetus and of cord, as well as by infectious diseases of the mother or of the fetus, or of both (such as, smallpox, pneumonia, typhoid and typhus fevers, influenza, and syphilis), by deformities and various toxemias and chronic diseases of the mother, and by injuries and accidents in pregnancy and labor.

From the material now under consideration, it is not possible, of course, to determine the relative importance of these various causes of stillbirth. It is well established that the effects of many of these causes may be modified or even completely controlled by appropriate care during pregnancy and labor. Hence, to a considerable degree, the proportion of stillbirths to live births in a community may be used as an indicator of the character of obstetrical care obtained by pregnant and lying-in women. When both the maternal and fetal death rates are high, unless the race stock is particularly weak, poor obstetrical care may be assumed. When the maternal death rate is comparatively low and the stillbirth rate is high, and vice versa, it may be predicated that obstetrical care is good but inadequately balanced. When the death rates for both mother and fetus are low, efficient and well rounded obstetrical care is indicated.

influence upon infant mortality. This is found in puerperal septicemia, the one prominent cause of maternal deaths from causes connected with the puerperal state that does not ordinarily act or begin to act until some days after labor.

CAUSES OF HIGH MATERNAL DEATH RATES AND HIGH STILLBIRTH RATES IN THE UNITED STATES, AND THEIR CORRECTION.

Abundant experience teaches that high maternal and infantile death rates from causes connected with childbearing are associated with inadequate care afforded by ignorant and careless accouchers and nurses and that, conversely, low death rates obtain in the practice of well-trained and careful attendants, even under environmental conditions ordinarily considered adverse. It is known that physicians and midwives of certain levels of intelligence and education can be trained to become accomplished accoucheurs. It is equally well known that in the United States, until very recent years, diploma mills ground out some intelligent and numbers of unintelligent and poorly trained men and women physicians with hopelessly inadequate obstetrical training and that many of these are still in the full bloom of practice. It is no secret that there are in this country no properly organized schools for training midwives and that even where midwife practice is supposedly regulated by legal statute, registration, examination, and supervision are tragically farcical. Of all the important branches of the nursing art, that of obstetrics is perhaps at once the most neglected and the most important. It cannot be questioned that the risks to both mother and child, even in the care of the most intelligent and well-trained accoucheurs, are enhanced by those constitutional weaknesses, racial or individual, inherited or acquired, general or local, that influence deformities, accidents of pregnancy and labor, toxemias, and resistance to infection. There is considerable evidence that the negro race in the United States is in these respects constitutionally weaker and therefore a poorer childbirth risk than the white. To what degree this handicap is increased by poorer obstetrical care is, however, unknown, while, in the indefinite congeries of causes grouped under accidents of pregnancy in Tables VI and XIII, the rates for whites are actually higher than for negroes; in accidents of labor, puerperal albuminuria and eclampsia, and septicemia, the rates for negroes are invariably higher, and for Baltimore, there is some evidence that these differences are to a certain extent independent of the character of obstetrical care. These data are, however, inadequate for far reaching conclusions.

Why should maternal mortality rates be so much lower in the rural than in the urban communities of the United States Birth registration area and in Kentucky, Maryland, North Carolina, and Virgina, and yet lower in the large cities of New York, Philadelphia, Boston, and Baltimore than in the rural districts, in only one of which cities, New York, is there evidence that administrative measures have influenced that result—and there only in the one category, puerperal fever? To what degree is the difference between rural and urban maternity death rates due to the carrying of the worst risks to "cities" for obstetrical care? What is the difference between the relative distribution of midwife and medical obstetrical care in urban and rural communities? Are midwives, on the whole, better and safer obstetrical attendants in the United States than physicians? Is either the average medical or midwife attendant in rural superior to those in urban communities? To what degree are the variations in the rates as ascertained influenced by the differences in the ages at which childbearing occurs, and in the number of pregnancies per woman, in urban and rural communities? Are so-called native-born women worse or better childbed risks than foreign-born women, and if so, why, and how do the latter vary in this regard among the various immigrant stocks? Are women living in rural districts inherently better obstetrical risks than women of the same race in cities, and are the lower rates obtaining in New York, Philadelphia, and Baltimore due to superior obstetrical care operating on material even less favorable than in the rest of the birth registration area? What are the most important preventable causes of stillbirth? These are some of the many important questions demanding answers.

Prevention and control of illness and death of mother and child are among the most neglected and potentially the most fruitful domains of American public health administration, and, of the problems concerned, the two greatest are the toxemias of pregnancy (including albuminuria and eclampsia) and puerperal fever, of which, the latter is the more readily approached.

My present purpose, however, of calling attention to the importance both to public health administrators and to scientific investigators of specific morbidity and mortality rates in general, and in the puerperal state in particular, of the use of a method of greater exactness than any in common use, is sufficiently served by the material presented.

To those whose duty it is to ascertain the causes and to apply the remedies, it may with propriety be pointed out that vital statistics holds the same relation to advances in public health knowledge and administration that pathological anatomy bears to the advance of fundamental knowledge in clinical medicine and surgery. In investigating the causes of death in the puerperal state, with but comparatively slight increase in work, in municipal and state health departments rates still more refined may be obtained by making subclasses under some of the rubrics—as for instance, in 134, accidents of pregnancy, certain deaths not concerned in childbirth could be eliminated. In administrative efforts directed toward reducing the mortality in the puerperal, including the pregnant, state, deaths from not only puerperal infection, but causes under all other rubrics, must be analyzed, both as to the environment and as to the obstetrical personel under which they occur in different kinds of women. It is likely that at least in the United States, the personal attendant is generally the most important factor. Those physicians and midwives who have consistently high maternal or infantile death rates would become evident, and could be dealt with appropriately. The information acquired in regard to the other causes will suggest suitable remedies.

In all such studies, it is of the greatest importance that careful consideration should be given to race, and by race is here meant race stock and not the deceitful classification by nationality as expressed by geography. If in a large city, the birth certificate be made to show the age of the mother and the number of previous children born live and dead, sufficient data would be accumulated in a short time to determine the relation of the number of children born, both singly and plurally, and the age of mothers to maternal and infantile mortalities. There is happily in the department of health of the city of New York evidence of a direct relation between the statistical studies in the Bureau of Records and the successful efforts toward the administrative control of puerperal fever, but in this respect New York stands in lonely isolation.

Among the lessons disclosed in this study is the deplorable lack of proper appreciation and use of statistical methods and of inductive reasoning from data elicited thereby, in the city and state health departments of the United States birth registration area. It is almost inconceivable that a community large or small, inhabited by people of average intelligence and of relatively great wealth, would fail to apply remedies for such glaring and wholesale women murder, as the data for the United States birth registration area, properly studied, disclose. When the horror of the conditions these data divulge is fully appreciated, it is much to be feared that as usual, the *opinions* of experts, mostly self-appointed, will be followed, with effects disastrous in life and money. The methods to be followed should be based not upon mere opinions drawn from hasty assumptions snatched from the clouds, but upon generalizations reached through inductions from adequate data analyzed, with the aid of appropriate statistical methods, by persons capable of consecutive thought.

SUMMARY.

It has been shown in this investigation that:

1. By using the sum of the living and the still births reported and the number of the deaths of women from causes connected with the puerperal state, rates are calculated expressing the actual risk of dying for women exposed to childbirth with a fair degree of specificity and much more accurately than those obtained by the methods in common use.

2. When this method is applied to the data for births and maternal deaths in the birth registration area of the United States for 1918, the total rate and the rates for causes under the important rubrics are conspicuously high. The total rate is more than double that for England and Wales calculated on live births alone, and this is due to the great excess in the United States' rates for puerperal albuminuria and convulsions and septicemia, and accidents of pregnancy and labor.

3. In the birth registration area of the United States for 1918, the total rate in urban communities was considerably greater (10 per cent.) than that in rural communities.

4. In the four states, Kentucky, Maryland, North Carolina, and Virginia, and in the cities, Washington, D. C., and Baltimore, Maryland the total rates and the rates for the most important rubrics are much higher for negro than for white women, and the negro rates are markedly in excess of the whole in both city and country. The rates for both white and negro women are higher in urban than in rural communities. The excess in total rates, in relation to both race and location, is due chiefly to higher rates for puerperal albuminuria and convulsions and septicemia.

5. As determined for the birth registration area of the United States for 1918, the age of mothers exerts a marked and progressive influence upon maternal mortality from causes connected with child-bearing.

6. As compared with Birmingham, England, and with Stockholm, Sweden, the maternal risk-rate in childbearing is much higher in New York, Philadelphia, Baltimore, Boston, and Washington, D. C., and in the order named. The total rates in these American cities are lower than the urban and rural total rates for the birth registration area of the United States for 1918.

7. The proportion of stillbirths to total births varies considerably in different places and in different races. In the birth registration area of the United States, the proportion for urban and rural communities is approximately the same, and the proportion for the negro is double that for the white, and for the urban negro somewhat greater than for the rural negro.

8. By intensive analysis of the data available in municipal and state health departments, it would be possible to determine with considerable accuracy to what degree some of the causes of high stillbirth rates and most of the causes of high maternal death rates in childbearing are due to poor obstetrical service on the part of physicians and midwives, the extent to which particular individuals are repsonsible for these fatalities, and the measures necessary to reduce them.

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THE BACTERIAL CONTENT OF TELEPHONES WITH SPECIAL REFERENCE TO RESPIRATORY PATHOGENS.*

By CLARENCE C. SAELHOF, M.S.

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Bissell (1) sets forth the question as to whether the public telephone transmits disease. A limited amount of work was performed with special reference to the *B. diphtheriae,* the conclusions of which were that it was impossible to demonstrate this organism on the transmitter. Other work has been done on the bacterial flora of telephones, particularly by Allan (2) who isolated *B. tuberculosis* from public call office booths, and by Tomarkin (3) who worked with pathogenic organisms (*B. diphtheriae, B. typhosus,* etc.) with special reference to their viability under varying conditions of exposure, disinfection, etc.; however, none of the articles discusses completely the bacterial content of the transmitter or receiver.

That the public telephone might be a causative factor in the transmission of certain respiratory diseases, for example, influenza, pneumonia, etc., either by direct contact (mouth of the individual placed immediately in the transmitter, or the receiver approximated directly to the ear), or by some other mode of transmission, led me to investigate the bacterial content of a series of telephones in Chicago.

Cultures from the transmitters and receivers of 94 telephones were taken at various periods as follows: 21 before the influenza epidemic (during the summer of 1919); 28 during the epidemic (the fall and winter of 1919–20); 24 following the epidemic (the first week of February, 1920), and 21 in a second post-epidemic group (the first week of March, 1920).

The telephones selected were all public, located in booths, usually in drug stores, cigar stores, etc., and were used by a large number of people each day. They were not taken from an isolated territory, but included large districts, both residential and factory. The telephones were divided roughly into three classes; "clean," in which the mouthpiece and booth seemed fairly clean; "filthy," in which a residue of dirt, grease, etc., was present in the transmitter and the booth none too clean; and "very filthy," in which the transmitted and receiver were covered by a thick layer of dirt, buccal and nasal secretions, etc.

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The manner of obtaining the organisms was as follows: Sterile throat swabs were dipped into sterile salt solution and replaced into too clean; and "very filthy," in which the transmitter and receiver were swabbed by encircling them three or four times. These swabs were returned to the laboratory, applied first to Loeffler's blood serum for the detection of the diphtheria bacillus, next to "chocolate blood agar" for the detection of the influenza bacillus, and lastly inserted into plain veal broth (pH 7.0, using phenol red as indicator), and incubated at 37° C. for ten hours. At the end of eight hours, the Loeffler blood serum tubes were examined for the diphtheria organism. The "chocolate blood agar" was allowed to incubate for forty-eight hours, being examined for all suspicious colonies in the meantime. One loopful of the ten hour growth in broth was inoculated into blood agar (12 drops of fresh rabbit's blood to every 5 c.c. of agar); plates were poured and then incubated for twenty-four hours.

Macroscopic with subsequent microscopic examinations of all colonies were made and, in suspicious cases, transplants made to obtain pure cultures.

The organisms encountered were not numerous. I was chiefly interested in those pathogenic for man. The types and percentages of organisms found during the different periods are listed in the accompanying Table.

TABLE I.

Percentage recovery of various bacteria during different periods.

Bacteria.	Preceding influenza epidemic. 21 telephones examined.	During influenza epidemic. 28 telephones examined.	Post-influenza epidemic, Feb. 1, 1920. 24 telephones examined.	Post-influenza epidemic, Mar. 1, 1920. 21 telephones examined.
Streptococcus hemolyticus	7.69%	4.00%	1.47%	2.75%
Staphylococcus hemolyticus	11.53%	9.00%	5.88%	6.25%
Staphylococcus aureus	3.85%	6.00%	5.88%	2.50%
Staphylococcus albus	32.69%	36.00%	41.17%	34.75%
Bacillus diphtheriae	0	2.00%	0	0
Diphtheroid bacillus	0	1.00%	0	0
Pneumococcus	0	1.00%	0	0
Bacillus coli	11.53%	6.00%	16.17%	12.50%
Bacillus subtilis	32.69%	35.00%	29.41%	41.25%

In all, 11 strains of hemolytic streptococci (Beta type) were recovered. On blood agar, they appeared as a sharp, pin point colony with a clear, distinct zone of hemolysis. Ten strains were virulent for rabbits. One strain was non-virulent, the animal surviving 33 days after an injection of 5 c.c. of a twenty-four-hour broth culture.

To determine virulence, broth suspensions of a twenty-four hour culture of the organism were injected into the marginal vein of the ear of young rabbits, weighing about 1000 grams. Arthritis and death usually occurred in from thirty-six to ninety-eight hours. Recovery of the organisms from the heart's blood and joints was usually possible. No attempt was made at differentiation by fermentation reactions.

Of the staphylococci encountered, a large number were hemolytic in character and retained that property throughout many transfers on artificial media. *Staphylococcus albus* was common. The colonies appeared large, white, moist and glistening, measuring 0.5 to 4 mm. in diameter; many were surrounded by a clear zone of hemolysis, in some cases distinct, in others, tending to fade out at the margins. Pathogenicity was not determined. *Staphylococcus aureus* was present in moderate numbers. They were hemolytic, but on artificial media soon tended to lose that property. One strain retained it for two months.

Two strains of diphtheria bacilli were isolated, both during the height of the influenza epidemic. Strain 7 was of the granular type, while Strain 10 was barred. .2 c.c. of broth suspensions of each were inoculated into guinea pigs. The animals died within fifty hours, necropsy revealing fibrinous exudation of fluid into the body cavities, with intense congestion of the viscera, especially the adrenals. Control animals were protected by antitoxin.

One strain of a diphtheroid bacillus was recovered. It resembled closely the true diphtheria organism, but was nonpathogenic for guinea pigs. According to Hamilton's (4) classification, it belongs to Group 1.

One strain of pneumococcus was found on a transmitter immediately after a person had finished conversation over the telephone. The organism was bile soluble, fermented inulin and possessed a distinct capsule.

Colon bacilli were present commonly in large numbers. They were identified by fermentation reactions. Bacillus subtilis was regarded as a contamination.

No influenza bacilli were isolated on the "chocolate blood agar."

Experimentally, the viability of the different types of organisms isolated was tried on transmitters under varying conditions. Ten mouthpieces were cleaned with soap and water until all debris had been removed, washed with alcohol and ether to remove the fatty material, and then immersed in a 1/1000 bichloride solution for three days. Ten other mouthpieces, filthy and more or less covered with mouth excretions, were taken directly from booths and swabbed in the manner described previously. The clean and filthy mouthpieces were set up side by side and tenacious, mucoid sputum, containing the types of organisms recovered, was planted on both and allowed to dry. Daily swabs were taken and plated on suitable media. The hemolytic streptococcus was recoverable after the longest period of time (four days) with the staphylococcus and pneumococcus in subsequent order. The organisms lived two to three days longer on the filthy transmitters than on the clean ones. The infectivity of a mouthpiece would therefore vary inversely with its cleanliness. This is in accord with facts already established.

In comparison with the transmitter, the receiver showed very few pathogenic organisms. This is as one might expect. Organisms may be implanted on the receiver from individuals suffering from a discharging ear. By intimate contact with the ear, as the receiver is during conversation, organisms could localize in the external auditory canal and under suitable conditions possibly set up an infection. An even greater danger might be the general dissemination of virulent organisms from running ears through contact of the receiver with the hand.

The telephones during the influenza epidemic contained more pathogenic organisms than during the interepidemic periods. However, hemolytic streptococci were more numerous preceding the epidemic than during or following it.

Numerous devices, as special mouthpieces, ear devices, etc., have been and are on the market to promote cleanliness. However, the following procedure appears to be of more practical value than any device now upon the market. The transmitter and receiver should be cleaned first by the use of good soap and warm water to remove the debris, and then sterilized in 1/1000 bichloride of mercury, lysol or some other disinfectant solution for a period of ten minutes; this to be done once or twice a week, with the daily removal of dust and secretions by means of cloths moistened in bichloride of mercury or some other antiseptic. The booths should be swept out at least once a week and disinfectants applied with subsequent proper ventilation.

From the above it is not to be concluded that the telephone plays even an important rôle in the transmission of infectious diseases. Indeed the data presented are of such character that no conclusions regarding frequency of transmission can be drawn therefrom. It is probable that the danger of infection from this source is slight. However the point may be emphasized that the telephone is an instrument upon which dangerous bacteria are commonly deposited and there continue to live for some time. This source of infected material should be known and as a possible danger under certain conditions should be given proper consideration.

SUMMARY.

1. Various pathogenic bacteria are present and can be isolated from the transmitters and receivers of telephones.

2. Hemolytic streptococci were isolated in 15.9 per cent., the diphtheria bacillus in 2 per cent., and the pneumococcus in 1 per cent., from the transmitters and receivers of 94 telephones.

3. 90.9 per cent. of 11 strains of hemolytic streptococci isolated were virulent for rabbits.

4. Sterilization of telephones should be practised to prevent the spread of virulent organisms. Cleansing with soap and warm water and subsequent sterilization in bichloride of mercury, lysol, etc., for a period of 10 minutes, is recommended.

5. In speaking, the mouth should not come in direct contact with the transmitter. The public should be taught how to use the telephone hygienically.

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THE VITALITY AND VIABILITY OF HEMOLYTIC STREPTOCOCCI IN WATER.*

By GEORGE S. LIVINGSTON, M.S.

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INTRODUCTION.

The subject of the viability and vitality of bacteria in water has been thoroughly investigated, mainly with reference to the bacterial content of natural waters to determine their potability. More especially has attention been centered upon *Bacillus coli* and its more dangerous relatives in the field of hygiene and public health, to the end of preventing and controlling the endemic and epidemic water-borne diseases. The scope of this work was extended to the streptococci, and the investigations here reported were conducted to ascertain their behavior and significance in water.

The early workers in this field reported on the mere presence of streptococci, with little attempt at classification. More recently the literature contains references to specific types which appear always to be of intestinal origin, and are usually non-hemolytic.

The object of the present work was to obtain data on the vitality and viability of hemolytic streptococci in water, and to discuss the possible rôle which various waters containing these organisms might play in the spread of disease.

The earliest reference to streptococci in water is that of Roscoe and Lunt (1) in 1891, who found them in sewage. Laws and Andrews (2), in 1894, found them in sewage from St. Bartholomew's Hospital. Houston (3) in 1898 reported the first of an extensive series of studies on streptococci in water. Classification on the basis of hemolysis was not used at that time, but it may be judged from the source and nature of the organisms, that they were of non-hemolytic varieties. In the United States the first reference to streptococci in water was made in 1902, by Winslow and Hunnewell (4) who found them in sewage, in septic tanks, and in river water polluted by sewage.

It appears, then, that streptococci have been found in water contaminated by excretions, and that where there is human fecal contamination the organism most commonly found is *Streptococcus fecalis,* a non-hemolytic variety. No reference can be found to hemolytic streptococci in water, but their occasional presence in feces suggests the possibility of finding them in water polluted by sewage. Holman (5) states that of fifty-three strains of streptococci from feces, thirteen were hemolytic, and that of thirty-eight strains from urine and the urethra, eleven were hemolytic. Oppenheim (5) found five hemolytic strains out of fifteen isolated from feces, and Broadhurst (7) reports that nine out of thirty-one fecal strains were hemolytic. Davis (8), however, found no hemolytic streptococci in the stools in fifty-three cases, many of whom harbored these organisms in their throats; he also found them absent from the stools of four scarlet fever patients. Moody and Irons (9) found them in the stools in thirty per cent. of cases of scarlet fever. It appears, therefore, that hemolytic streptococci are rare and inconstant inhabitants of the excretions, and that the probability of their reaching water by this route is slight. That disease may be spread by water containing streptococci is suggested, however, by Wilson (10), who reports an epidemic of streptococcus pneumonia with intestinal complications, in a war camp. All of the patients gave a history of having drunk shell-hole water, the diarrheal symptoms ensuing from one to five hours after. Bacteriological examination of the water showed that twenty-eight out of forty-two samples contained streptococci in large numbers. Hemolytic activity was variable, but it appears that some of the strains isolated were hemolytic.

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Hemolytic streptococci appear not to have a natural habitat outside the animal body. Broadhurst (7) examined eighteen samples of soil and water from wood edges, moist road-banks, and brooks, of which one sample of water, from a country roadside overflow, showed short-chained micrococci; these, however, were not hemolytic.

Experimental work.

The experimental work of the problem comprised a series of tests to determine the vitality and viability of hemolytic streptococci in water, under various conditions. In these tests the general method employed was to place the water in a suitable vessel in measured amount, and seed it with a suspension of hemolytic streptococci. These were grown in pure culture on slants of blood-agar for forty-eight hours, at 37.5° C. The growths were then removed by scraping the surfaces of the slants with a wire loop, and transferred to a tube of sterile distilled water. This gave a concentrated suspension of the bacteria in water, without admixture of any nutrient material from the culture medium. The suspension was shaken until it was of uniform opacity, and contained no large particles, and was then added to the water in Erlenmeyer flasks of Pyrex glass, to give the desired concentration. In those tests in which enumeration of the bacteria was not necessary, a qualitative examination was made at the time of seeding, and periodically thereafter, once a day, once in two days, or twice a week, as the conditions of the experiment required. For this purpose plates of blood-agar were used, inoculated with the water, and incubated at 37.5° for twenty-four hours. The plates were examined for the presence of hemolytic streptococcus colonies, and if found, the water was recorded as containing living hemolytic streptococci on the day on which the sample was taken. Negative plates were incubated for twenty-four hours longer, before recording them as such.

In certain experiments it was necessary to keep a daily record of the quantitative streptococcal content of the water. This was done by pouring blood-agar plates inoculated with accurately measured amounts of the water by the method of decimal dilutions. After the usual incubation, colonies were counted in the most suitable plate, and the number of hemolytic streptococci per cubic centimeter of water was calculated.

I. *Longevity of various strains of hemolytic streptococci in sterile distilled water.*

Eleven strains of hemolytic streptococci and one of *Streptococcus viridans* were selected for comparative study. All the strains were of human origin, and all were virulent at the time of isolation. All but two of the strains had caused the death of the individual from whom they had been isolated, and these two were associated with acute infectious processes. The strains varied in age from one month to ten years. They were obtained from the following sources:

1. Abscess (autopsy).
8. Acute ulcerated appendix.
12. Lung; influenzal pneumonia (autopsy).
39. Lung; pneumonia (autopsy).
40. Tonsils; acute tonsillitis.
84. Trachea; pneumonia (autopsy).
90. Heart blood; septicemia (autopsy).

104. Pleura; pneumonia (autopsy).
113. Lungs; pneumonia (autopsy).
208. Cerebrospinal fluid; streptococcus meningitis.
211. Fatal septic sore throat; during milk epidemic.
49. Streptococcus viridans; endocarditis (autopsy).

All the strains grew in chains of varying length, in liquid media; all were Gram positive; all grew in characteristic colonies on blood-agar plates, with a clear wide zone of hemolysis around the colony (except 49); none of them liquefied gelatin, and none fermented inulin. Each strain was added to 100 c.c. of sterile distilled water, in a cotton-plugged flask, by the method previously outlined, and the flasks were kept in the laboratory, at room temperature, in subdued light. The presence of hemolytic streptococci was determined qualitatively only, by plating on blood-agar. Plates were poured at the time the experiment was begun, and twice a week thereafter, until negative plates appeared. Negative plates on three consecutive days, using one cubic centimeter of water, were taken as evidence that all streptococci in a given flask of water were dead.

TABLE I.

Longevity of various strains of hemolytic streptococci, in sterile distilled water.

Days.	104	12	113	49	40	39	90	84	211	8	208	1
Start	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	−
10	+	+	+	+	+	+	+	+	+	+	+	
14	+	+	+	+	+	+	+	+	+	+	+	
17	+	+	+	+	+	+	+	+	+	+	+	
21	+	+	+	+	+	+	+	+	+	+	+	
24	+	+	+	+	+	+	+	+	+	+	−	
28	+	+	+	+	+	+	+	+	+	−		
31	+	+	+	+	+	+	+	+	−			
35	+	+	+	+	+	+	+	−				
38	+	+	+	+	+	+	−					
42	+	+	+	+	+	−						
45	+	+	+	+	+							
49	+	+	+	+	−							
52	+	+	+	−								
56	+	+	−									
59	+	+										
63	+	−										
66	+											
70	+											
73	+											
77	+											
80	+											
84	+											
87	+											
91	−											

The results of this experiment show that the length of life of these organisms in water, under these conditions varies within wide limits. It is, in effect, a test of the resistance of each strain to starvation, since no nutrient material was present in the water. Strain 1 was the first to succumb, no colonies appearing in plates after the third day. Strain 104 survived the longest, viz., eighty-seven days. The average length of life of all the hemolytic strains was thirty-eight days. The green-producing streptococcus, 49, lived for fifty days. The average for all strains was thirty-nine days. In Table I the strains are arranged in the order of their longevity.

II. *Variation of longevity of a single strain of hemolytic streptococci, in different concentrations, in sterile distilled water.*

A heavy suspension was made of strain 104, from a number of blood-agar slants. This was adjusted to contain approximately one billion streptococci per cubic centimeter. The suspension was added to flasks of sterile distilled water, in quantities to give a final content of 100 c. c. of water for each flask, the bacterial content varying as follows:

1. 100,000,000 per c.c.
2. 10,000,000 per c.c.
3. 1,000,000 per c.c.
4. 100,000 per c.c.
5. 10,000 per c.c.
6. 1,000 per c.c.

These flasks were kept in the laboratory, at room temperature, in subdued light. Quantitative estimations of the bacterial content were made at the start of the experiment, and at two day intervals thereafter. Absence of colonies in plates, poured from 1 c. c. of water, on three successive days, was taken as evidence that all streptococci in a given flask were dead. Table II gives the detailed record of this experiment. The flasks are arranged in the order of their longevity. The bacteria in Flask I survived for fifty-two days, in Flask 2 for thirty days, in Flask 3 for twenty days, in Flask 4 for twelve days, in Flask 5 for six days, and in Flask 6 for four days. The results were quite uniform and significant. The greatest number of organisms died during the first week. After that the rate of decrease was less rapid. The greater the concentration of streptococci in a given flask of water, the longer they survived. The curve below shows the relation between the initial concentration of streptococci in water and their longevity.

TABLE II.

Variation in longevity of a single strain of hemolytic streptococci, in different concentrations, in sterile distilled water.

Days.	1	2	3	4	5	6
	No. per c.c.	No. per c.c.	No. per c.c.	No. per c.c.	No. per c.c.	No. per c.c.
Start	100,000,000	10,000,000	1,000,000	100,000	10,000	1,000
2	40,000,000	6,000,000	910,000	6,300	451	29
4	9,000,000	1,500,000	250,000	945	77	3
6	800,000	500,000	72,000	124	8	0
8	300,000	95,000	13,700	38	0	
10	150,000	36,000	2,150	14		
12	90,000	8,500	890	6		
14	35,000	2,300	315	0		
16	22,000	650	84			
18	12,000	435	27			
20	4,500	210	6			
22	1,700	62	0			
24	1,100	18				
26	845	4				
28	670	7				
30	595	3				
32	230	0				
34	180					
36	195					
38	65					
40	23					
42	14					
44	3					
46	4					
48	5					
50	4					
52	2					
54	0					

III. *Longevity of a single strain of hemolytic streptococci in various waters.*

Samples of water were collected from various sources, and a suspension of strain 104 was added to each sample, in a flask. 100 c. c. of each kind of water were used, and the streptococcal content was adjusted to approximately 100,000 per c. c. The flasks were kept in the laboratory, at room temperature, in subdued light. The tests for viability were qualitative only. Blood-agar plates were poured daily. The routine technic was used. In addition, tubes of 1 per cent. dextrose bouillon were inoculated, and smears made after incubation for twenty-four hours. These were stained by Gram's method, and examined for streptococci. This was useful in those cases where the blood-agar plates were doubtful, owing to excessive contamination by other organisms. The sources of the various waters and the longevity of the streptococci, in each, are listed below.

1. Sterile distilled water	15 days
2. Sterile physiological salt solution	12 "
3. Water from deep well	11 "
4. Water from surface well	8 "
5. Water from Lake Michigan	7 "
6. Street water, sterilized in autoclave	6 "
7. River water, Desplaines River	6 "
8. Water from country road-side ditch	5 "
9. Street water, not sterile	4 "
10. Water from park lagoon	4 "
11. Tap water	3 "
12. River water, Chicago River	2 "

The average survival in all waters was seven days.

It is to be noted that these waters can be roughly divided into two groups, viz.:

A. The "clean" group, comprising those waters in which vitality was retained for the average length of time or longer, and

B. The "dirty" group, comprising those waters in which the streptococci died out in less than seven days. An exception is No. 11, tap water, in which all organisms were dead after three days. The experiment was later repeated, with sterile distilled water, as a control. The results were substantially the same, the streptococci dying sooner in tap water, in each case. This is attributed to the fact that the tap water used was quite heavily chlorinated. In all of the dirty waters other bacteria, the natural contaminators, were present in large numbers, after all streptococci were dead. It would seem that this was the main factor in determining the length of their vitality, they having been crowded out and overgrown by the saprophytes present. In Table III the water specimens are arranged in the order in which the streptococci survived.

IV. *Effect of temperature on rate of decrease.*

In this experiment three flasks, each containing 300 c.c. of sterile distilled water were seeded with a suspension of Streptococcus hemolyticus, strain 211, adjusted to approximately 100,000 per c.c. One flask was kept in the incubator at 37.5° C., another at room temperature, in subdued light, the average being 27° C., and the third

TABLE III.

Longevity of a single strain of hemolytic streptococci in various waters.

Days.	1	2	3	4	5	6	7	8	9	10	11	12
Start....	+	+	+	+	+	+	+	+	+	+	+	+
1......	+	+	+	+	+	+	+	+	+	+	+	+
2......	+	+	+	+	+	+	+	+	+	+	+	+
3......	+	+	+	+	+	+	+	+	+	+	+	−
4......	+	+	+	+	+	+	+	+	+	+	−	
5......	+	+	+	+	+	+	+	+	−	−		
6......	+	+	+	+	+	+	+	−				
7......	+	+	+	+	+	−	−					
8......	+	+	+	+	−							
9......	+	+	+	−								
10......	+	+	+									
11......	+	+	+									
12......	+	+	−									
13......	+	−										
14......	+											
15......	+											
16......	−											

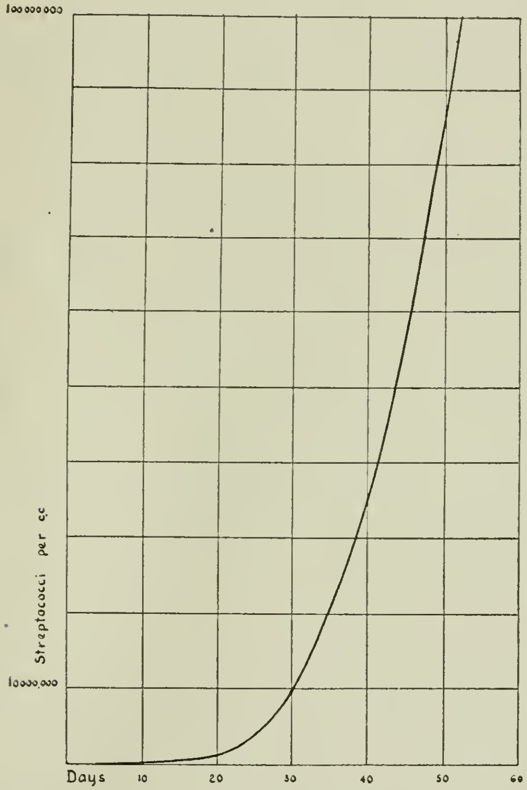
was kept in the refrigerator, at an average temperature of 1° C. Quantitative plates were poured daily, and the number of surviving streptococci, per cubic centimeter, was calculated. Strain 211 was one isolated during a milk epidemic from a fatal case of streptococcus sore throat. It was an old laboratory strain having been isolated ten years previously. In the incubator the streptococci re-

TABLE IV.

Effect of temperature on rate of decrease.

Days.	37.5° C.	27° C.	1° C.
	No. per c.c.	No. per c.c.	No. per c.c.
Start........	100,000	100,000	100,000
1..........	23,000	18,500	37,000
2..........	3,400	7,200	15,700
3..........	655	1,170	4,200
4..........	48	415	1,740
5..........	9	167	885
6..........	2	38	390
7..........	0	6	187
8..........		15	93
9..........		4	43
10..........		0	21
11..........			27
12..........			16
13..........			4
14..........			7
15..........			3
16..........			0

tained their vitality, in water, for six days, at room temperature for nine days, and in the refrigerator for fifteen days. It appears, then, that survival is favored by low temperature, and is hindered by high temperatures. Too much importance, however, must not be attached to these figures, since they are not widely separated, and the differences are not sufficiently pronounced to be of much significance. Table IV gives the detailed record of these tests. It is to be noted that during the first week there was a rapid decrease in all three flasks. The rate of decrease is much less for subsequent days. The rapid decrease in the first week is quite constant, and appears to be characteristic.



Curve showing relation of the concentration of streptococci to their longevity in water.

V. *Comparison of longevity of virulent and avirulent strains of hemolytic streptococci, in sterile distilled water.*

A number of strains of hemolytic streptococci was selected for this study, some from old laboratory cultures, others, recently isolated, from various human sources. Rabbits were inoculated with these strains to determine their virulence. 1000 gram rabbits were used. Each was given three cubic centimeters of a twenty-four hour broth culture of the strain used, injecting into the ear vein. Ten avirulent strains were chosen, viz., such as produced no change in the animals in two weeks; and ten virulent strains, each of which had produced arthritis and caused death in the rabbit inoculated. Each strain was

TABLE V.

Comparison of longevity of virulent and avirulent strains of hemolytic streptococci, in sterile distilled water.

Days.	Avirulent strains.										Virulent strains.									
	7	6	11	4	3	5	10	8	2	12	20	23	28	21	22	24	25	26	27	31
Start...	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.....	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
2.....	+	+	+	+	+	+	+	+	+	+	+	+	+							
3.....	+	+	+	+	+	+	+	+	+	+	+	−	−							
4.....	+	+	+	+	+	+	+	+	+	+	+									
5.....	+	+	+	+	+	+	+	+	+	+	+									
6.....	+	+	+	+	+	+	+	+	+	+	+									
7.....	+	+	+	+	+	+	+	+	+	+	−									
8.....	+	+	+	+	+	+	+	+	+	+										
9.....	+	+	+	+	+	+	+	+	+	+										
10.....	+	+	+	+	+	+	+	+	+	+										
11.....	+	+	+	+	+	+	+	+	+	−										
12.....	+	+	+	+	+	+	+	+	+											
13.....	+	+	+	+	+	+	+	+	−											
14.....	+	+	+	+	+	+	+	−												
15.....	+	+	+	+	+	+	−													
16.....	+	+	+	+	+	+														
17.....	+	+	+	+	+	−														
18.....	+	+	+	+	−															
19.....	+	+	+	+																
20.....	+	+	+	+																
21.....	+	+	+	−																
22.....	+	+	+																	
23.....	+	+	+																	
24.....	+	+	−																	
25.....	+	+																		
26.....	+	+																		
27.....	+	+																		
28.....	+	+																		
29.....	+	+																		
30.....	+	−																		
31.....	+																			
32.....	+																			
33.....	−																			

seeded in 100 c. c. of sterile distilled water, as in the previous tests, and kept at room temperature in the laboratory. Qualitative determinations were made daily. In Table V the various strains are arranged, in the order of their longevity. The avirulent strains varied in their survival from ten to thirty-two days, averaging over eighteen days. Of the virulent strains one survived for six days, two for two days, and all the rest succumbed within a day of the start.

Discussion.

In reviewing the results of the series of experiments set forth above, one is struck by the wide variations and the inconstancy in the behavior of hemolytic streptococci in water. Any conclusions which are drawn from this work must, therefore, be very general.

It is seen that hemolytic streptococci are capable of retaining their vitality when kept in water. In Experiment I the average period of survival was thirty-eight days, but one strain, 104, was able after being kept for eighty-seven days under these conditions, to multiply rapidly, when placed in a favorable environment. That the long period of survival was due in part to the enormous numbers originally placed in the water, is shown by the behavior of the same strain in various concentrations in Experiment II. Of a small number of streptococci in water, all will die out rapidly, but of a larger number a few individuals retain their vitality for a considerable length of time. Houston (3) tested a large number of streptococci in sterile tap water and sterile salt solution. Many died out in a few days, and those, that survived for forty or fifty days, showed a great decrease in their number.

Under more natural conditions the element of interference by other organisms becomes an important factor. Thus in waters of the "dirty" group, in Experiment III, hemolytic streptococci failed to survive for more than a week. Savage and Wood (11) found that streptococci of the intestinal variety die rapidly in sewage, and that most of them are dead at the end of two weeks.

In Experiment IV it was found that the rate of death, in water, is higher at body temperature than at room temperatures, and is lowest at 1° C. The differences in the periods of survival at the various temperatures were not marked enough to be of great significance. Hinds (12), working with colon and typhoid bacilli, in natural and distilled water, found that the rate of death increased with the temperature.

The greatest diminution in numbers was found in the first week. Those organisms which survived longer were eliminated more gradually. This agrees with the findings of Savage and Wood (11) with fecal streptococci. They conclude that the finding of streptococci in water is evidence of recent pollution.

Hemolytic streptococci, stored in water, always progressively decrease in their numbers, as shown by several of the quantitative tests. Savage and Wood found the same to be true of fecal streptococci, but noted that colon bacilli increased in number for a considerable length of time.

The effect of virulence is striking. In Experiment V none of the virulent strains survived as long as the least viable of the avirulent strains. Most of them died out over night. The evidence is quite definite that virulent hemolytic streptococci, recently isolated from lesions in the human body, are poorly adapted to storage in water, while old strains, long accustomed to artificial media, survive much longer.

Summary.

Hemolytic streptococci, when placed in water, remain alive for a variable length of time, depending upon their number, upon the temperature, upon the presence of other organisms, and upon virulence. They are capable under special conditions, of retaining their vitality for a long time, but under natural conditions, if placed in water, they will succumb quite rapidly, especially if recently isolated.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL. XIX. ON THE INFLUENCE OF FREE CHOICE OF FOOD MATERIALS ON WINTER EGG PRODUCTION AND BODY WEIGHT.*

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INTRODUCTION.

From extensive and careful observation of the behavior of the domestic fowl the senior author has long held the opinion that current practice in the feeding of poultry is, from a theoretical point of view at least, biologically unsound. The methods of feeding poultry advocated by the recognized authorities in that branch of husbandry are essentially mass or average methods, while appetite and vital performance of whatever sort are biologically highly individualistic matters. Some years ago the following statement was published regarding the matter (1): " The successful feeding of poultry depends upon experience and acquaintance with fowls. The basic biological factor is, once more, individuality. Each individual hen is an independent living thing, possessing well marked likes and dislikes of her own with respect to food. There can be no question that the best results in the way of egg production and meat production would be obtained if a skillful feeder could feed each individual fowl by and for itself. Evidence that this is the case is found in the fact, which is universal wherever poultry is kept, that on the average fowls kept in small flocks, of say under 25 birds each, do relatively much better than larger flocks. The production and money returns per bird are greater. The fundamental reason for this is that the birds in small flocks get better care as individuals. When a man has only such a small number to take care of he can recognize their individual peculiarities more easily. Furthermore an individual bird stands a better chance of having its peculiar taste gratified in a small than in a large flock."

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* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 25.

The experiments here recorded were carried out at the Maine Agricultural Experiment Station during the autumn and winter of 1916–17. The entry of the United States into the war broke off the work. It has been completed and prepared for publication by the senior author.

It may be that mass or average methods of feeding poultry are economically preferable to individualistic methods, but there is no real evidence that such is the fact, for the simple reason that no adequate data on the point have ever been collected. Such experiments as have hitherto been made in feeding poultry in ways which would permit of free individual choice of food materials on the part of the birds have dealt with small numbers.

The purpose of the present investigation was to determine, with a sufficiently large number of birds, to give a statistically significant result, and selected with extreme care to secure genetic uniformity between controls and experimental birds, what effect on fecundity would be produced by permitting birds free choice as to kind and amount of food eaten, in comparison with others fed by the usual mass methods. Incidentally it was possible to make observations on a number of other interesting points.

Pioneer work in the field of animal husbandry on the subject of free choice feeding was done by Evvard (2) with hogs. His first experiment was to give the pigs an opportunity to choose the amount of salt to be used in their diet. A basal ration was fed to five lots of pigs and after a check period of ninety days, one lot was allowed no salt; the second lot received 18 grams of salt daily; the third lot received 36 grams of salt daily; and the fourth lot received 72 grams of salt daily. In each of the above instances where salt was fed it was mixed with the basal ration "of 6.1 parts corn to 1 part linseed oilmeal." The fifth pen received the basal ration, but the pigs were allowed free access to the salt. The results of this experiment in Evvard's own words were:

"The requirement for a hundred pounds of grain was comparatively less in the 'salt ad libitum' group (Lot V) than where the salt was omitted (Lot I), or mixed with the feeds (Lots II, III, and IV). It is quite evident that the addition of salt in varying results, in every instance, is a cheapening of the gain; the feed required for a hundred pounds was lessened. It is quite evident that the pigs which received salt according to their appetite made relatively the most economical gains of any group. The free choice group compared to the 'no salt group' in the 71-day salt feeding period required only 89.36 in contrast with 101.88 per cent. as much feed as in the preliminary."

In the second experiment "three groups of five pigs each were fed in dry lot practically from weaning time until they were more than eight months of age." In each pen the pigs were allowed to select their diet and to eat as much as they wanted, and to eat at any time they wished, for food was always before them. A careful record was kept of the food consumed, and the ration eaten compared to rations compounded according to the "Illinois or Dietrich Standard" and the "Wolff-Lehmann standard."

Among the very interesting points brought out by the above experiment are:

1. That a "very marked changeableness of the pigs' appetites is manifested by what they eat throughout the period of growth and fattening." During the growing period the young pig consumes a larger amount of "high protein feeds," but when growth slackens the desire changes and the pig consumes a greater amount of the "carbohydrate-fat" feeds; or to put it in another way "as the pigs unfold in their development they vary their intake of food nutrients."

2. In the second place, greater development was brought about by the "free-choice" method of feeding than has been secured by any other method of feeding.

3. It would seem that the palatability of the foods varied with the degree of development in the animal.

Jackson (3) performed an experiment of this sort with poultry. The hens were kept in separate pens, one individual by itself in each pen, and fed foods placed in separate holders. The results of the three experiments reported show a marked individual preference of food on the part of the birds used. In the first experiment the foodstuffs consumed in the largest amount were as follows, named in the order of decreasing amounts: Wheat, corn, oats, mash; the second experiment showed a different order—namely: Wheat, corn, mash, oats; and the third experiment showed still a third order: Corn, wheat, oats, mash, On examination of the figures given it is seen that in the third experiment the wheat was only slightly less in amount than the corn consumed. The other two experiments conformed closely to the results here reported.

A similar experiment to that of Jackson was carried on by Kempster (4). He used only sixteen hens, each one having a separate pen, and with the food placed before them in cans. The purpose of the experiment was, among other things, to " study individual preference for certain foods " and " to observe changes in their food selection while laying and when not."

It was found that wheat was preferred above all the other food presented, the birds eating more than two and a half times as much wheat as any other one grain. There was noticed, however, a variation in the tastes of the individual birds, for " five consumed more kafir " than wheat; " one consumed more middlings;" while another consumed " more corn meal," which together with Evvard's experiment indicates that the system of feeding a ration made up of only one or two kinds of food to a group of animals or a flock of hens is utterly insufficient to meet the needs of all of the individuals which go to make up the group or flock. It was also found that the birds when laying consumed a ration having a narrower nutritive ratio than when they were not producing eggs.

Material and methods.

The experiment began September 21, 1916, when the pullets were taken from the range to winter quarters in the long laying house. In order that all conditions, save the food, should be the same one of the large compartments of the laying house was divided by a wire partition. The litter on the floor throughout the entire experiment was made up of soft wood shavings.

The two pens were designated as A and B. Pen A was known as the experimental pen and Pen B as the check pen, and the birds in the latter were given the regular Maine Station ration. Great care was taken, in selecting the pullets, to have those in Pen B as nearly identical to those in Pen A as was possible. In making the selection an equal number of birds accustomed to a given chick ration were placed in each pen, and when a new breed or cross-bred combination was introduced an equal number of individual representatives of the breed was placed in each pen.

On September 21, 1916, 25 pure bred Barred Plymouth Rock pullets hatched April 2, 1916, were put in Pen A, and a like number in Pen B. Four pullets* were added to each pen on October 10. The next

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* For exact information as to breeds see Table I.

TABLE I.

Showing date, number and breed of birds put in Pens A and B.

Pen A.		Date.	Pen B.	
No. of birds.	Breed.		No. of birds.	Breed.
25	B. P. R.	Sept. 21	25	B. P. R.
2	B. H. × Wh. Leg.	Oct. 10	1	B. H. × Wh. Leg.
2	B. P. R. × B. H.	Oct. 10	3	B. P. R. × B. H.
9	B. P. R. × Hdn.	Oct. 11	9	B. P. R. × Hdn.
1	B. P. R. × Hdn. × B. H.	Oct. 11	1	B. P. R. × Hdn. × W.
6	B. P. R. × B. H.	Oct. 11	5	B. P. R. × B. H.
2	Golden Barred	Oct. 11	2	Golden Barred
1	Extracted White	Oct. 11	1	Extracted White
1	W. W. × E. W.	Oct. 11	1	B. P. R.
1	B. P. R. × Hdn.	Oct. 12	1	B. P. R. × Hdn.
1	W. P. R.	Oct. 12	1	W. P. R.
1	W. W.	Oct. 12	1	W. W.
1	B. P. R.	Oct. 12		
1	Bh. × Wh. Leg.	Oct. 20	1	B. B. × Wh. Leg.
1	B. P. R. × B. H.	Oct. 20	2	B. P. R. × B. H.
2	B. H. × Wh. Leg.	Nov. 7	3	B. H. × Wh. Leg.
2	B. P. R. × B. H.	Nov. 7	2	B. P. R. × B. H.
2	B. P. R.	Nov. 11	2	B. P. R.
61			61	

Key to above table:
B. P. R. = Barred Plymouth Rock.
B. H. = Black Hamburg
Wh. Leg. = White Leghorn
Hdn. = Houdan
W. W. = White Wyandotte
E. W. = Extracted White
Hdn. W. = White Houdan

day, October 11, twenty pullets were put in Pen A and nineteen in Pen B, and the following day, October 12, four were added to Pen A and three to Pen B. On October 20 two pullets were added to Pen A and three to Pen B. The only loss from death occurred on October 28 when No. 296 from Pen A died, death being caused by a pathological condition of the ovary. In November 7 three pullets were added to Pen A and five to Pen B, and on November 11 two birds were added to each pen, making a total of 60 birds in Pen A and 61 in Pen B or 121 birds used throughout the experiment.

The following materials were given as food:

Pen A.

Wheat,
Cracked corn,
Oats,
Bran,
Red-dog flour,
Linseed meal,
Bone meal,
Meat scrap,
Shell,
Grit,
Charcoal,
Green food.*

Pen B.

Cracked corn,
Wheat,
Oats,
Dry mash,†
Shell,
Grit,
Green food.*

Bran	300 lbs.	Middlings	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.

The composition of the mash varied each month as follows:

From September 1 to October 1:

Bran	300 lbs.	Middlings	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.

From October 1 to November 1:

Bran	200 lbs.	Middlings	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.

From November 1 to December 1:

Bran	200 lbs.	Gluten meal	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.
Middlings	100 lbs.	Linseed meal	50 lbs.

From December 1 to January 1:

Bran	200 lbs.	Gluten meal	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.
Middlings	100 lbs.		

From January 1 to March 1:

Bran	200 lbs.	Gluten meal	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.
Middlings	100 lbs.	Linseed meal	50 lbs.

* The green food varied from time to time as various materials were available. From September 21 to October 9 the green food was in the form of cut corn; from October 9 to December 10 cabbage was given; from December 10 to December 13 mangel beets were used; and from December 13 to the close of the experiment sprouted oats were given.

† The dry mash given was the regular Maine Experiment Station dry mash consisting of:

In Pen A the foods except the green food were placed in separate compartments of specially built hoppers, which were kept well filled so that the hens had access to food at all times of the day. The green food when in the form of cut corn and sprouted oats was thrown on the floor, and when in the form of cabbage and mangels was hung on a nail within easy reach of the pullets. After the hoppers had once been put in place they were never changed and throughout the experiment they contained the same kind of food. All of the hoppers used were of the same size, and with one exception, were divided into two equal compartments each holding a different kind of food. One hopper was divided into three compartments and held the grit, oyster shell and charcoal in the respective parts.

In Pen B the green food was fed in the same manner as in Pen A. The wheat, oats and cracked corn, mixed together, were given as a scratch food and thrown in the litter twice a day, morning and at noon. The dry mash was fed in a long hopper which was filled every morning. The grit and oyster shells were mixed and offered in one hopper during the first part of the experiment, but after November 10 they were fed in separate hoppers. The charcoal was mixed with the dry mash.

An accurate record was kept of all the food that was put into the hoppers, and after the first period, which was from September 21 to October 21 inclusive, the hoppers were cleaned every Friday morning. All of the material found in the hoppers at that time was carefully weighed, and the amount subtracted from the total amount put in during the week gave the amount consumed by the hens during that period. After the hoppers had been cleaned fresh food was placed in them so that after the first period the birds received fresh food every Friday morning. This procedure reduced the chance of the food becoming mouldy or stale.

During the first period birds were being added from time to time, and consequently they were in a more or less disturbed state, and time was allowed for them to become accustomed to the new environment.

Every twenty-eight days the birds were weighed individually and the measurement of the width of the pubic bones taken. In this way we were able to acquaint ourselves with the monthly gain or loss of each bird.

At the end of each feeding period the data collected were arranged in suitable tables and the net amount of food consumed, as well as the number of grams of food consumed per bird per day; the number of grams of food used in the production of one egg, and the percentage of each food eaten to the total amount consumed were determined.

In determining the nutritive ratio, the ration made up of the food eaten during each period was used as a basis, and not the amounts eaten each day. In determining the nutritive ratio of the ration eaten by Pen B in which the dry mash composed the larger part it was first necessary to find the sum of the respective digestible nutrients of the several constituents of the mash. As we were unable to find any publications giving the digestion coefficient for sprouted oats it was necessary to use, in determining the nutritive ratio of the ration, the digestible nutrients of whole oats. The percentages of available nutrients used for sprouted oats were determined by Mr. James M. Bartlett, Chemist of the Maine Experiment Station, especially for this work, using oats having sprouts about four inches long. In making the determination the entire plant, both stem and roots, was used. We are greatly indebted to Mr. Bartlett for this aid.

In figuring the nutritive ratio of the various rations the following formula was used:

$$\frac{\text{Carbohydrates} + \text{Fat} \times (2.25)}{\text{Protein}}.$$

It will be seen that the fiber was not taken into consideration.

In the case of food materials fed from hoppers it is possible to determine accurately the amount actually consumed by the birds, because one can weigh in an amount at the beginning of a period, weigh out what is left at the end, and the difference is the amount consumed. The hoppers were so constructed as to prevent the birds scattering and wasting the food in the process of feeding from them. In the case of free-choice Pen A the consumption of all materials fed except green food could be accurately determined in this way. In the case of Pen B the only materials for which accurate consumption figures could be got in this way were dry mash, shell and grit. The whole grains, wheat, oats, and cracked corn, were in Pen B, as already explained, scattered on the litter and the birds scratched for them. Consequently the figures in that case, just as in all statistical estimates of human food consumption (5) represent consumption plus undeterminable waste. Careful as the feeder may be there will always be a certain amount of grain left in the litter when fed in this way.

In the case of green food the same condition obtained in both pens. When the green food is in the form of cabbage or mangel-wurzel the consumption can be accurately determined, because the uneaten residue can be weighed. When sprouted oats are used, however, a certain undeterminable amount gets trampled in the litter and lost. So that here again figures given represent consumption plus wastage. In respect of this food, however, both pens were on precisely the same footing.

FOOD CONSUMED.

In Tables II and III are set forth the amounts of each kind of food consumed in each period by each pen of birds.

TABLE II.

Net kilograms of food consumed in each period. Pen A.

Period.	Wheat.	Cracked corn.	Oats.	Bran.	Flour.	Corn-meal.	Lin-seed meal.	Bone-meal.	Meat Scrap.	Shell.	Grit.	Char-coal.	Green food.
1....	95.91	29.55	20.80	6.06	.61	13.59	.00	1.00	11.54	4.45	1.84	1.63	134.49
2....	22.00	12.38	4.31	.11	.48	2.45	.05	.59	1.22	.88	.50	.05	30.05
3....	21.64	9.32	4.76	2.86	.39	3.72	.00	.50	1.86	.86	.84	.09	29.48
4....	21.82	8.98	4.58	.64	.39	5.17	.00	.32	1.81	.95	.61	.00	24.95
5....	19.69	7.08	3.63	.64	.50	5.22	.00	.07	1.72	.86	.91	.05	25.51
6....	21.95	6.42	3.08	.91	.64	4.97	.00	.27	1.63	1.04	.91	.02	17.46
7....	21.41	7.80	5.83	1.18	.54	4.60	.00	.14	1.86	1.02	.73	.05	17.69
8....	26.97	8.23	8.12	.57	.66	5.35	.00	.18	1.22	1.13	.88	.02	16.06
9....	19.96	8.89	7.89	.64	.41	3.63	.00	.25	1.02	.95	1.09	.05	14.52
10....	17.37	12.93	8.98	1.09	1.13	2.27	.00	.27	2.09	1.22	1.00	.05	9.19
11....	19.82	11.07	10.05	.64	.91	5.33	.00	.09	1.66	1.32	.82	.09	11.34
12....	19.16	10.36	8.03	.54	.43	6.26	.00	.05	1.77	1.27	1.52	.09	12.81
13....	23.07	8.75	5.53	.54	.61	4.63	.00	.00	1.27	1.66	.86	.07	12.47
Totals	350.77	141.76	95.59	16.42	7.70	67.19	.05	3.73	30.67	17.61	12.51	2.26	356.02

TABLE III.

Net kilograms of food consumed in each period. Pen B.

Period.	Cracked corn.	Wheat.	Oats.	Dry mash.	Shell.	Grit.	Green food.
1.........	50.35	15.24	18.69	100.15		5.08	122.79
2.........	12.70	7.62	5.08	27.67		.59	25.51
3.........	12.70	7.62	5.08	27.01		.95	21.55
4.........	12.70	7.62	5.08	27.13	1.45	1.95	16.67
5.........	12.70	7.62	5.08	27.49	1.04	1.41	18.48
6.........	12.70	7.62	5.08	29.39	1.18	3.18	13.83
7.........	12.70	7.62	5.08	29.39	1.18	1.18	13.38
8.........	12.70	7.62	5.08	32.39	.59	2.04	13.38
9.........	12.70	7.62	5.08	30.21	1.09	1.07	14.06
10.........	12.70	7.62	5.08	27.13	1.72	1.13	10.09
11.........	12.70	7.62	5.08	29.21	1.72	1.63	11.34
12.........	12.70	7.62	5.08	28.21	1.81	.54	12.81
13.........	12.70	7.62	5.08	26.76	1.68	1.32	12.93
Totals.....	202.75	106.68	79.65	442.14	13.46	22.07	306.82

For these tables the following points are to be noted:

1. Considering first the whole grains, the " free-choice " birds ate more than three times as much wheat as the mass-fed birds ate and wasted; the former also ate somewhat more oats than the total consumption and wastage of the latter. Of cracked corn the consumption and wastage of the mass-fed birds was greater than the consumption of the "free-choice" birds. Taking totals of all "scratch-feeds" (whole grains) the Pen A (free-choice) birds ate during the whole experiment 588.12 kgs. against 389.08 kgs. of the same feeds consumed and wasted by the Pen B (mass-fed) birds.

2. The reverse relation obtained in respect of ground materials (mash). In total Pen A (free-choice) birds consumed during the whole experiment 128.02 kgs. (including bran, red-dog flour, cornmeal, linseed meal, bone meal, meat scrap, and charcoal) against 442.14 kgs. of the same materials in the form of dry mash consumed by the Pen B birds. In other words, when the birds were given free choice of their food about 12 per cent. of the ration was made up of milled products (including meat and charcoal) while the mass-fed birds took about 38 per cent. of their total ration in this form.

3. A noteworthy point is the small amount of beef scrap eaten by the " free-choice " birds. It has often been observed that birds eating a dry mash in which beef scrap was a constituent part, would pick out the bits of meat, and it was expected that birds having free access to beef scrap would avail themselves of the opportunity and eat a large amount. This, however, was not the case, for the total amount of beef scrap eaten amounted to 30.67 kilograms which is 2.78 per cent. of the ration. This observation is similar to that noted by Jackson (*loc. cit.*) who states: "The low consumption of meat scrap was something of a surprise. None of the hens in any of the tests . . . displayed any particular liking for this foodstuff, though the brand used was considered the most palatable that could be obtained." Kempster (*loc. cit.*), likewise found that the birds when allowed to choose their own ration ate only a small amount of beef scrap, and concluded that " the comparatively small consumption of beef scrap indicates that hens eat only enough to satisfy their demands."

4. The birds in Pen B consumed more grit and oyster shell than the "free-choice" birds, even though they were fed, in this respect, in nearly the same way. Except for the first three periods, grit and oyster shell were fed in separate hoppers, as in Pen A. During the

first three periods the grit and shell were mixed and fed in the same hopper. It will be noticed that the total amount of grit and shell consumed in Pen B is 35.53 kgs. while the "free-choice" birds ate only 30.12 kgs. The difference in consumption of these ingredients is probably correlated with the differences already noted in the composition of the grain portion of the rations consumed.

5. The amount of green food eaten shows that the "free-choice" birds consumed somewhat more than the birds in Pen B. The green food was measured out to both pens in nearly the same amounts at first, but it was noticed that the "free-choice" birds ate the green food much more eagerly and apparently with greater relish, so that the amount was slightly increased. They consumed in total 256.02 kgs., which is 32.30 per cent. of all they ate, while the birds in Pen B consumed 306.82 kgs., which is 26.14 per cent. of the total consumption of that pen.

6. Although bran is considered as a very necessary part of all rations fed to poultry, the "free-choice" birds ate very little—only 16.42 kgs., which is less than one per cent. of the total amount. Only a very small amount of red-dog flour was eaten, due probably to the fact that as soon as it is taken into the mouth it forms a dough which is swallowed only with difficulty. Cornmeal made up 6.10 per cent. of the ration, but bone meal was less than one per cent.

TABLE IV.

Per cent. of total amount of food consumed. Pen A.

Period.	Wheat.	Cracked corn.	Oats.	Bran.	Flour.	Corn-meal.	Linseed meal.	Bone meal.	Meat scrap.	Shell.	Grit.	Charcoal.	Green food.
1..	29.83	9.19	8.47	1.88	.19	4.23	0	.27	3.59	1.38	.57	.51	41.83
2..	29.31	16.49	5.74	.15	.63	3.26	.06	.79	1.63	1.18	.66	.06	40.03
3..	29.19	12.07	6.17	3.70	.50	4.82	0	.65	2.41	1.12	1.09	.012	38.18
4..	31.07	12.79	6.52	.90	.05	.74	0	.05	2.58	1.36	.87	.0	35.53
5..	29.88	10.74	5.51	.96	.76	7.92	0	.10	2.62	1.31	1.38	.07	38.74
6..	37.02	10.82	5.20	1.53	1.07	8.37	0	.46	2.75	1.76	1.53	.04	24.44
7..	34.06	12.41	9.27	1.87	.87	7.33	0	.22	2.96	1.62	.12	.07	28.14
8..	38.85	11.86	11.69	.82	.96	7.71	0	.26	1.76	1.63	1.27	.03	23.13
9..	33.66	14.99	13.31	1.07	.68	6.12	0	.43	1.72	1.60	1.83	.07	24.48
10..	30.16	22.46	15.59	1.89	1.97	3.94	0	.47	3.62	2.13	1.73	.08	15.95
11..	31.40	17.53	15.92	1.01	1.44	8.44	0	.14	2.62	2.08	1.29	.14	17.96
12..	30.76	16.63	12.88	.87	.69	10.04	0	.07	2.84	2.03	2.43	.14	20.56
13..	38.78	14.72	9.30	.92	1.82	7.78	0	0	2.13	2.78	1.45	.10	20.98

The percentages of total consumption for the two groups are shown in Tables IV and V.

TABLE V.

Per cent. of total amount of food consumed. Pen B.

Period.	Cracked corn.	Wheat.	Oats.	Dry mash.	Shell.	Grit.	Green food.
1.........	16.12	4.88	5.98	32.07		1.63	39.32
2.........	16.04	9.63	6.42	34.95		.74	32.23
3.........	16.95	10.17	6.78	36.06		1.27	28.76
4.........	17.47	10.49	7.00	37.36	2.00	2.68	22.96
5.........	17.20	10.32	6.88	37.23	1.41	1.90	25.03
6.........	17.40	10.44	6.96	40.27	1.62	4.35	18.95
7.........	18.00	10.80	7.20	41.67	1.67	1.67	18.97
8.........	17.20	10.32	6.88	43.88	.80	2.76	18.13
9.........	17.68	10.61	7.07	42.05	1.54	1.48	19.58
10.........	19.39	11.63	7.76	41.42	2.63	1.72	15.41
11.........	18.32	10.99	7.33	42.14	2.48	2.36	16.36
12.........	18.46	11.07	7.38	41.01	2.64	.79	18.63
13.........	18.65	11.19	7.46	39.31	2.46	1.93	18.98

TABLE VI.

Net kilograms of digestible nutrients consumed in each period. Pen A.

Period.	Protein.	Carbohydrate.	Fat.	Calories.
1.............	21.013	110.022	6.867	601107
2.............	4.685	25.097	1.299	134187
3.............	5.229	26.597	1.246	142074
4.............	4.868	25.992	1.349	139072
5.............	4.387	23.049	1.193	123583
6.............	4.345	23.298	1.267	125119
7.............	4.673	24.922	1.320	133615
8.............	5.166	28.166	1.461	150249
9.............	5.803	28.983	2.019	161399
10.............	5.427	27.315	1.997	152814
11.............	6.025	33.822	2.108	182977
12.............	5.691	33.224	2.111	179184
13.............	5.336	32.128	1.831	170631
Totals.........	82.648	442.615	26.068	2396011

TABLE VII.

Net kilograms of digestible nutrients consumed in each period. Pen B.

Period.	Protein.	Carbohydrate.	Fat.	Calories.
1.............	26.358	91.679	8.452	562555
2.............	7.737	25.656	2.297	158273
3.............	7.980	25.163	2.278	157072
4.............	7.673	24.976	2.278	155046
5.............	7.782	23.299	2.298	148803
6.............	8.057	25.593	2.390	160192
7.............	7.784	25.703	2.353	159180
8.............	8.358	27.670	2.573	171644
9.............	8.879	32.693	3.044	198754
10.............	7.972	29.821	2.697	180033
11.............	8.612	30.790	2.884	188369
12.............	8.555	31.023	2.903	189268
13.............	8.388	30.826	2.881	187571
Totals.........	124.135	424.892	39.328	2616760

We may next consider the nutrient composition of the rations consumed. The data are given in Tables VI, VII, VIII, IX, and X.

TABLE VIII.

Grams of digestible nutrients per bird per day. Pen A.

Period.	Protein.	Carbohydrate.	Fat.	Calories.
1	15.43	80.78	5.04	441.33
2	12.36	66.22	3.42	353.98
3	13.41	68.20	3.19	364.27
4	11.64	62.18	3.23	332.70
5	10.45	54.88	2.84	294.27
6	10.34	55.47	3.02	297.91
7	11.13	59.34	3.14	318.13
8	12.30	67.06	3.48	357.74
9	13.82	69.01	4.81	384.34
10	12.92	65.03	4.75	363.77
11	14.35	80.53	5.02	435.69
12	13.55	79.10	5.03	426.64
13	12.83	77.23	4.40	410.17
Totals	164.53	885.03	51.37	4780.94

TABLE IX.

Grams of digestible nutrients per bird per day. Pen B.

Period.	Protein.	Carbohydrate.	Fat.	Calories.
1	19.70	68.52	6.32	420.48
2	20.47	67.87	6.07	418.65
3	20.30	64.03	5.80	399.69
4	18.05	58.76	5.36	364.77
5	18.66	54.56	5.38	350.24
6	18.87	59.94	5.59	375.11
7	18.23	60.19	5.51	372.77
8	19.57	64.80	6.02	401.90
9	20.79	76.56	7.13	465.44
10	18.67	69.84	6.31	421.57
11	20.17	72.11	6.75	441.12
12	20.04	72.65	6.79	443.18
13	19.65	72.19	6.74	439.23
Totals	253.17	862.02	79.77	5314.15

From these tables it is evident that:

1. Not only did the "free-choice" birds consume wholly different proportions of the several sorts of foodstuffs, but the net nutrient composition of their ration was widely different from that of the ration consumed by the mass-fed controls.

2. The general nature of the difference in rations may best be indicated by taking the results of the experiment as a whole. The Pen A birds consumed in the whole period 33.4 per cent. *less* protein and 33.7 per cent. *less* fat than did the Pen B birds. The carbohydrate consumption was 4.2 per cent. *more* in Pen A than in Pen B. Finally,

TABLE X.

Nutritive ratios of rations consumed.

	Nutritive ratios.	
Period.	Pen *A*.	Pen *B*.
1	1:6.0	1:4.2
2	1:6.0	1:4.0
3	1:5.6	1:3.8
4	1:6.0	1:3.9
5	1:6.0	1:3.7
6	1:6.0	1:3.8
7	1:6.0	1:4.0
8	1:6.1	1:4.0
9	1:5.8	1:3.9
10	1:5.9	1:4.5
11	1:6.4	1:4.3
12	1:6.7	1:4.4
13	1:6.8	1:4.4

the total energy intake in calories of the Pen A birds was 8.4 per cent. lower than that of controls in Pen B. It need not be emphasized that these differences in the two rations in respect to protein, fat, and calories, are relatively large.

3. In terms of nutritive ratio the birds of Pen A operated on approximately a 1:6 ratio against a ratio of 1:4 in Pen B. Put in another way, in the case of Pen A birds about 15 per cent. of the ration was protein, about 80 per cent. carbohydrate, and about 4.5 per cent. fat, against about 21 per cent. protein, 72 per cent. carbohydrates, and 6.5 per cent. fat in the case of Pen B birds.

4. The above results are clearly not of an accidental average character but the differences in ration between the two sets, which have been pointed out, were maintained with marked consistency and regularity in each of the 13 periods. Indeed, the general smoothness and uniformity of the results is noteworthy and bears evidence to the critical care with which the experimental work was done.

5. In general, the nutritive intake tended to increase during the last half dozen periods. This is in accordance with expectation in view of the cold weather and increasing egg production.

With such extreme differences in nutrient intake one would naturally expect comparable differences in energy and material output in the form of eggs, and in body weight changes. We may next examine the facts in regard to these matters.

Egg production.

The basic data regarding the egg production of these birds are given in Table XI, and are summarized in Table XII.

TABLE XI.

Showing daily egg production and the number of eggs per 100 birds per day.

Date.	Pen A.		Pen B.	
	Daily egg production.	No. of eggs per 100 birds per day.	Daily egg production.	No. of eggs per 100 birds per day.
September 20	0	0	0	0
21	4	16	8	32
22	4	16	9	36
23	6	24	6	24
24	10	40	7	28
25	7	28	5	20
26	12	48	6	24
27	6	24	7	28
28	5	20	6	24
29	8	32	5	20
30	10	40	9	36
October 1	8	32	8	32
2	7	28	4	24
3	8	32	8	32
4	7	28	8	32
5	8	32	10	40
6	11	44	8	32
7	9	36	4	16
8	10	40	9	36
9	14	56	12	48
10	10	34.5	5	17.2
11	10	20.4	10	20.8
12	14	26.4	10	19.6
13	11	20.7	11	21.6
14	14	26.4	13	25.5
15	12	22.5	13	25.5
16	11	20.7	12	23.5
17	15	28.3	15	29.4
18	13	24.5	13	25.5
19	11	20.7	8	15.7
20	15	27.3	14	25.9
21	14	25.5	13	24.1
22	10	18.2	15	27.8
23	7	12.7	10	18.5
24	16	29.1	19	35.2
25	15	27.3	13	24.1
26	14	25.5	11	20.4
27	16	29.1	15	27.8
28	11	20.4	11	20.4
29	18	33.3	13	24.1
30	16	29.4	16	29.6
31	17	31.5	14	25.9
November 1	14	25.9	11	20.4
2	10	18.5	13	24.1
3	15	27.8	13	24.1
4	17	31.5	14	25.9
5	16	29.4	11	20.4
6	12	22.2	15	27.8
7	20	34.5	15	25.4
8	13	22.4	12	20.3
9	13	22.4	11	18.6
10	16	27.6	13	22.0
11	19	31.7	18	29.5
12	21	35.0	15	24.6

TABLE XI (*Continued*).

Date.	Pen A.		Pen B.	
	Daily egg production.	No. of eggs per 100 birds per day.	Daily egg production.	No. of eggs per 100 birds per day.
November 13...	17	28.3	15	24.6
14...	20	33.3	16	26.2
15...	18	30.0	15	24.6
16...	20	33.3	18	29.5
17...	24	40.0	16	26.2
18...	19	31.7	15	24.6
19...	15	25.0	22	36.0
20...	23	38.3	14	22.9
21...	17	28.3	17	27.9
22...	24	40.0	20	32.8
23...	22	36.7	15	24.6
24...	19	31.7	21	34.4
25...	17	28.3	17	27.9
26...	19	31.7	16	26.2
27...	18	30.0	18	29.5
28...	22	36.7	17	27.9
29...	17	28.3	13	21.3
30...	16	26.7	18	29.5
December 1...	21	35.0	18	29.5
2...	16	26.7	18	29.5
3...	21	35.0	16	26.2
4...	16	26.7	18	29.5
5...	20	33.3	20	32.8
6...	12	20.0	14	22.9
7...	19	31.7	17	27.9
8...	19	31.7	20	32.8
9...	17	28.3	19	31.1
10...	20	33.3	17	27.9
11...	21	35.0	19	31.1
12...	17	28.3	21	34.4
13...	20	33.3	17	27.9
14...	14	23.3	19	31.3
15...	21	35.0	23	37.7
16...	19	31.7	17	27.9
17...	17	28.3	21	34.4
18...	16	26.7	19	31.1
19...	20	33.3	21	34.4
20...	21	35.0	19	31.1
21...	16	26.7	18	29.5
22...	24	40.0	22	36.1
23...	20	33.3	21	34.4
24...	21	35.0	16	26.2
25...	22	36.7	27	44.3
26...	18	30.0	22	36.1
27...	19	31.7	19	31.1
28...	19	31.7	24	39.3
29...	19	31.7	22	36.1
30...	21	35.0	23	37.7
31...	20	33.3	21	34.4
January 1...	22	36.7	23	37.7
2...	18	30.0	24	39.3
3...	22	36.7	19	31.1
4...	24	40.0	22	36.1
5...	18	30.0	20	32.8
6...	24	40.0	29	47.5

TABLE XI (*Continued*).

Date.	Pen A.		Pen B.	
	Daily egg production.	No. of eggs per 100 birds per day.	Daily egg production.	No. of eggs per 100 birds per day.
January 7...	24	40.0	17	27.9
8...	23	38.3	26	42.6
9...	21	35.0	20	32.8
10...	27	45.0	22	36.1
11...	18	30.0	20	32.8
12...	24	40.0	26	42.6
13...	19	31.7	17	27.9
14...	22	36.7	20	32.8
15...	18	30.0	20	32.8
16...	18	30.0	21	34.4
17...	15	25.0	20	32.8
18...	20	33.3	20	32.8

TABLE XII.

Number of eggs produced per pen per period.

Period-	Pen *A*.	Pen *B*.
1	366	344
2	102	93
3	106	91
4	131	110
5	144	119
6	128	120
7	125	121
8	128	132
9	130	138
10	143	151
11	146	154
12	155	154
13	136	144
Totals	1940	1871

These results in respect of egg production are striking, when taken in connection with the results on food consumption. We note:

1. The "free-choice" and mass-fed pens produced substantially the same number of eggs during the experiment, there being a slight excess, amounting to roughly 4 per cent., in favor of the free-choice birds.

2. Both pens gave a very good winter egg production, amounting to an average of over 30 eggs per bird with nearly a month and a half (January 19 to March 1) yet to go at the close of the experiment in order to complete the normal winter period (6). This fact is of importance as showing the general normality of the birds and their egg production under the experimental conditions.

3. During the first 7 periods the Pen A birds laid slightly more than the Pen B birds, while during the last 6 periods the relation was reversed, the differences in both cases being insignificant statistically.

Taking totals it appears that for each egg produced there was consumed, on the average, the nutrients indicated in Table XIII.

TABLE XIII.

Nutrients consumed by the flock per one egg produced.

Nutrient.	Pen A.	Pen B.	Per cent. increase Pen B over Pen A.
Protein	42.6 grams	66.4 grams	+55.8
Carbohydrates	228.2 "	227.1 "	− .5*
Fat	13.4 "	21.0 "	+56.4
Calories	1235.0 "	1398.4 "	+13.2

From this table it appears that the mass-fed birds consumed approximately 56 per cent. more of protein and of fat per egg produced than did the "free-choice" birds. The same amount of carbohydrate was consumed per egg in both cases. The energy intake associated with the production of one egg was 13.2 per cent. greater in Pen B than in Pen A.

It is apparent that the whole machinery of the domestic fowl is run much more economically in respect of energy and material intake when the birds are allowed freely to choose their ration from among the common poultry feeds, than under as good a system of mass-controlled feeding as has yet been devised. Whether this difference is specific for the reproductive portion of the machine we shall consider in the next section.

Body weight.

The birds used in this experiment, as has already been pointed out, were pullets. When they were brought in from the range and put in the laying house for the experiment their growth in body was not completed, though they were, as the egg records show, reproductively mature. They were weighed at monthly intervals throughout the experiment, with results shown in Table XIV.

It is at once clear from this table that:

1. The birds were properly selected for the experiment. At the first weighing the difference in mean body weight between the experimental and the control birds amounted to only .08 kg., an obviously insignificant difference.

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* Decrease.

TABLE XIV.

Average weight in kilograms of birds on days weighed.

Date.	Pen *A*.	Pen *B*.
Sept. 20	2.01	1.94
Oct. 27	2.39	2.27
Nov. 24	2.45	2.34
Dec. 22	2.52	2.44
Jan. 19	2.54	2.52

2. At the end of the experiment (actually 12 days before the final end) the two groups were only .02 kg. apart in mean body weight. In other words, in spite of the 30 per cent. lower intake of protein and fat on the part of the Pen A birds they were able to maintain equality of body weight with the Pen B birds, just as they have been shown above to have maintained equality of egg production. This can only mean markedly superior physiological utilization of the ration in the former case as compared with the latter.

Some general observations on body weight in relation to exercise are of interest. The birds in Pen B exercised more extensively than did those in Pen A because they found it absolutely imperative to do so, else they needs must forego the part of their daily meal made up by the scratch food. The birds in Pen A were by no means idle. During the day they would be found at almost any time, scratching and searching diligently in the litter as though they felt that perhaps there was a chance of finding a stray kernel of grain. The two hens that made the greatest gains were in Pen B where they had to scratch for their corn, wheat, and oats. Number 284 weighed 2.88 kgs. at the beginning of the experiment and 3.90 kgs. at the close, thus registering a gain of 1.02 kgs. She was the heaviest bird in the two lots. Number 320 of Pen A weighed 2.88 kgs. when put into the pen, and 3.72 kgs. at the close of the experiment, making a gain of .84 kg. The greatest gain made by any one bird was made by No. 298, a Barred Plymouth Rock pullet of Pen B, which weighed 2.25 kgs. at the beginning of the experiment (September 21), and 3.70 kgs. at the close of the experiment (January 19), registering a gain of 1.45 kgs. or .4 kg. per month. While the individual cases just mentioned are interesting they would be of little value as evidence for the entire flock in each pen, but the gains made by the flocks bear the same relation one to another. When put in the house the birds in Pen A weighed 110.81 kgs., and at the last weighing, after having lost one bird by death, the flock weighed 152.25 kgs., which is a gain of 41.44

kgs. The birds in Pen B weighed 104.49 kgs. when put in, and on January 19 at the last weighing they weighed 153.52 kgs, which is a gain of 49.03. Thus it would seem that the theory that birds will take on excessive weight if fed by the hopper method is not substantiated. Jackson got the same result, and has the following to say regarding the matter (*loc. cit.*, pp. 230–231). "It was feared that under the conditions of exclusive hopper feeding and close confinement, the hens would suffer from insufficient exercise. It is not possible to say what might have been the result of more liberty or a different system of feeding, but . . . there is no reason to think that the health of any was particularly affected by the conditions."

From the data as to total gain in weight and total nutrient consumption Table XV has been computed.

TABLE XV.

Nutrients consumed in association with the production of 55 grams gain in body weight by the flock.

Nutrient.	Pen A.	Pen B.	Per cent. increase pen B over pen A.
Protein..............	109.5 grams	139.1 grams	27.0
Carbohydrate.........	587.4 "	476.9 "	−18.8*
Fat..................	34.7 "	44.1 "	27.1
Calories.............	3180.0 "	2935.7 "	− 7.7*

We see here, just as in the case of egg production, that the free-choice birds made a much more economical utilization of their ration than did the control birds. For substantially the same gain in body weight the former group consumed 27 per cent. less protein and fat. The energy intake was, however, 7.7 per cent. more in Pen A than in Pen B. This arose from the carbohydrate relation. While the percentage difference between the two groups in respect of carbohydrate consumption was not large the absolute amount consumed is so large in proportion to protein and fat as to dominate the calory percentage.

The average weight of pullet eggs of the strain of birds on the Maine Station plant has been shown by Pearl and Surface (7) to be 55.264 gr. per egg. We have, therefore, taken 55 grams as the unit of the body weight table (Table XV). If we then compare the nutrient consumption associated with the production of the same amount (mass) of material as (*a*) egg and (*b*) body tissue, by the

* Decrease.

same birds at the same time, it is seen that, in general, something over twice as great average nutritive intake appears upon the unit of body-weight base, as upon the unit of egg-production base. It must, however, be remembered that the average of both Tables XIII and XV are inclusive. Egg production and growth in body-weight were both going on at the same time in the birds, while XIII and XV in a sense assume that all of the food intake went to a particular function only. The really only valid use of the figures of Tables XIII and XV, which can be made, is for comparing of the Pen A with the Pen B birds.

The nutrient intake of a laying pullet is used for the following purposes:

a. The maintenance and operation of the whole physiological machine as a going concern.
b. Growth (increase in body weight).
c. Production of eggs.
    1. Material and energy content of eggs themselves.
    2. Energy required to operate the egg-producing machinery.

Of these items *c*, 1 (the material and energy content of the produced eggs) can alone be separately determined. Using Atwater and Bryant's factors we have for the total eggs laid by the two groups the results shown in Table XVI.

TABLE XVI.

Nutrients in all eggs produced during experiment.

Nutrient.	Pen *A*.	Pen *B*.
Protein	14.045 kg.	13.545 kg.
Fat	9.971 "	9.616 "
Calories	150,315	144,963

It is at once apparent, that so far as actual output of material and energy in the form of eggs is concerned there is no essential difference in the two groups. Hence it must follow that the Pen B birds made a much less economical use of their nutritive intake in respect of the other physiological processes (*a, b,* and *c* 2 of the classification above) than did the birds of Pen A. If we subtract the figures of Table XV from those of Tables VI and VII we get the results shown in Table XVII.

TABLE XVII.

Total nutrients consumed for maintenance and operation of all physiological processes except output of eggs (net nutrient content of eggs produced deducted from gross total intake).

Nutrient.	Pen A.	Pen B.	Percentage increase of pen A over pen B.
Protein	68.603 kg.	110.590 kg.	+61.2*
Carbohydrate	442.615 "	424.892 "	− 4.0*
Fat	16.097 "	29.712 "	+84.6
Calories	2,245,696	2,471,798	+10.1

From this table it is seen how enormously more efficiently the Pen A birds utilized their nutrient intake for the maintenance of their general physiological economy than did those of Pen B. The case is like that of an inefficiently stoked as compared with a well stoked steam boiler fire. One can get the same energy output from the plant with a poor fireman as with a good, both using the same kind of coal, but only at the expense of a greatly increased coal consumption.

Discussion.

The broad, outstanding result of this work is that a flock of 60 pullets, when given entire freedom as to choice of their ration from among the common whole and milled grains, produced somewhat more eggs and made substantially the same gain in body weight as an equal group of birds fed according to one of the best standard methods of poultry feeding, and accomplished these results with a net nutrient consumption of 33 per cent. less protein, 34 per cent. less fat, and 8 per cent. smaller total energy intake. This is a remarkable result, and may have highly important consequences for the economic management of domestic poultry. It should be emphasized that save in respect of freedom of choice of food, both flocks were managed in exactly the same manner. They had equal floor space in identically the same pens, the same amount of water, and in every other possible particular except the feeding were treated in the same way. There was nothing peculiar or troublesome in the management. The management of large commercial flocks of poultry in the same way that our Pen A was treated is entirely practical and feasible, and will indeed involve far smaller labor costs than any other system of management yet devised. Whether it will be economically desirable to do this depends mainly on the relative costs of the different whole and milled grain products at the time. In this question we are not now interested.

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* Decrease.

A word of caution should be noted by anyone thinking to repeat these experiments. In order to get clear-cut results with poultry in an experiment of this kind many years' experience has convinced the senior author that a degree of knowledge of the genetic or hereditary characteristics of the individual birds is requisite, particulary in respect of fecundity, which is not likely to be possessed by any poultry farmer, and by but extremely few experimental plants. By a haphazard selection of experimental and control birds in respect of their genetic fecundity characters one might easily get results which would be widely divergent from those here reported. In this experiment the genetic constitution of every bird in respect of fecundity was thoroughly known, on the basis of individual pedigrees extending over many generations. The birds were so selected as to make the two pens genetically identical in respect of this important character. We should consider no experiment an adequate repetition of this one, whatever its results, in which it could not be demonstrated that this genetic identity between experimental and control birds was maintained. Most of the experimental results on feeding poultry, and other live stock for that matter, are, in a critical scientific sense, of very doubtful validity, just because the genetic factor always involved is so completely neglected. The senior author has come to feel that really before almost any sort of animal experimentation is undertaken it is essential that a stock be prepared of known and stable genetic constitution, by the necessary amount of controlled pedigree breeding, often requiring years of time. The debonair fashion in which physiological experimenters, using "physiological" in the broadest sense, draw conclusions from experiments often most elaborate in character, but made upon animals purchased from the animal dealer, about the innate individual constitutional peculiarities and differences of which the experimenter can possibly know nothing, gives one to wonder afresh at the naïve simplicity of the human animal.

One other point: Most of the experiments hitherto carried out with poultry upon the "cafeteria" mode of feeding have been done by putting single individual birds in pens or coops alone, each by itself. From extensive observations on the normal psychology of the domestic fowl, we doubt the significance of results got in this way. The hen is a gregarious creature, and is rather profoundly and apparently permanently upset by being forced to live alone. One does not look for normal results in respect of such subtle psychological matters as appetite under these conditions. Furthermore if the experiments are ever to have any practical meaning for poultry husbandry they must duplicate in some degree at least the practical conditions under which poultry is kept commercially.

SUMMARY.

The experiment here reported was designed to test what the effect would be upon egg production and gain in body weight of permitting domestic fowls to choose freely for themselves what kinds and amounts of food materials they would eat. An experimental flock of 60 birds was allowed free access to some 12 different common food materials presented to them at all times in hoppers, while a control flock, selected to be as nearly as possible of identical genetic constitution in respect of fecundity, was fed according to the system normally used at the Maine Agricultural Experiment Station, and found by years of experience there and in the hands of practical poultry men to give excellent results.

The chief results were:

1. The free-choice birds ate more of whole grain and less of milled products than the mass-fed birds.

2. The free-choice birds consumed 33.4 per cent. less protein, 33.7 per cent. less fat, and had a total energy intake in calories 8.4 per cent. smaller than the corresponding consumption (including wastage) of the mass-fed birds. The consumption of carbohydrate was somewhat, but not greatly higher in the free-choice birds.

3. With food consumption differences indicated above, the egg production, which in absolute amount was excellent, and the gains in body weight, were substantially identical in the two groups.

4. The conclusion appears justified that when fowls are allowed complete freedom of choice in the kind and amount of the food they ate, they make a better physiological utilization of their rations than under a system of man-controlled mass feeding, however excellent theoretically the system may be.

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CERTAIN MINIMUMS AND MAXIMUMS IN TUBERCULOSIS CONTROL.

By D. B. ARMSTRONG, M. D.,

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(Received for publication, February 1st, 1921.)

The Framingham Community Health and Tuberculosis Demonstration has now been under way for approximately four years. While this period is not of sufficient duration to justify final conclusions with reference to the ultimate preventability of tuberculosis infection, morbidity, and mortality, it has been a sufficiently long and intense experience to suggest tentative conclusions as to certain factors prominent, medically and socially, in the tuberculosis problem.

It is impossible to give here the details of the program and activities.* It is the intention of this paper rather to discuss certain of the less popular but fundamentally significant findings, based primarily on the observations made possible by relatively extensive medical machinery.

The Demonstration has made every effort to find tuberculosis, to treat the disease, to educate against its spread, and to cure known cases. To these ends it has used all available means, including popular and medical education, a diagnostic consultation service, thorough examination work in infant clinics, schools and factories, home and sanatoria treatment, etc. All this work has been carried out always with the generous aid of the local Framingham agencies, official and private. Indeed, the program has been developed with the object of making permanent in the community those activities demonstrated to be of worth, and either transferred to or built up under the auspices of local agencies.

During this period the Demonstration has had under observation or treatment a total of 366 cases, including arrested as well as active cases, classifiable as follows:

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* See Framingham Monographs Nos. 1 to 8 inclusive, special reports and reprints—available at the Community Health Station, Framingham, Mass.

Total active (early, advanced, convalescent):			
At home	41		
Away (institutions)	13		
		54	
Total arrested:			
From the active columns			
In Framingham	53		
Moved away	4		
Always in arrested column			
In Framingham	67		
Moved away	29		
		153	
Total cured		9	
Other moved away cases:			
In active column		32	
Deferred cases:			
In Framingham	20		
Moved away	9		
		29	
Dead		89	
Total			366

The above table, taken from the newly devised current classification chart in Framingham, gives a picture of the status of tuberculosis cases on January 1, 1921. The number of active cases, the number of cases being treated in institutions, the number of arrested cases, the number of cured cases, and the number of deaths in the entire group are shown therein. The phrase "moved away" covers cases that have either disappeared entirely or have gone to a distant point, such as Colorado, California, home to Italy, etc., and are no longer under the direct therapeutic observation of the Framingham office. The "deferred" cases are cases that when originally discovered were thought for a certainty to be suffering from tuberculosis. A further study with X-ray and other observation has thrown some doubt on the original diagnosis, and these cases are for the time being held in the deferred or suspicious group.

On the basis of a careful study of this representative group of tuberculosis cases, over a period of several years, the following tentative standards have been set up, any of which further experience may modify:

1. *The minimum number of cases in the average community that will probably escape detection in an early stage of the disease, in spite of reasonably adequate machinery.*

The relatively extensive medical machinery for the detection of tuberculosis in Framingham insures a fairly careful current observation of most of the age groups in the population. At the beginning of the Demonstration, before this machinery was in operation, 55 per cent. of the total cases reported during one year were recorded as suffering from tuberculosis in an early stage. During the last three years this percentage of the total reported cases discovered in an early stage has been increased to 71.

On the other hand, taking the entire group of cases under observation, including the arrested and unreported group, as well as the active reported cases, it is found that during the Demonstration period as a whole approximately 83 per cent. have come under observation or treatment in an early or arrested stage. This means that 17 per cent. were discovered as advanced. A careful study of these advanced cases indicates that an additional 3 per cent. should have been discovered in an early stage if the medical machinery in Framingham had been working smoothly in all respects. This leaves, therefore, a residue of 14 per cent. that would probably under practically any circumstances have escaped early detection. In most communities at the present time about 50 per cent. of the cases are discovered as advanced. On the basis of the Framingham experience it would seem that the application of fairly complete disease-detecting devices might be expected to reduce this percentage to 14, if not to a lower figure.

2. *The minimum number to die in a representative group of tuberculous individuals.*

During the four year period, out of a total of approximately 360 tuberculous individuals 21 per cent. have died. A study of these deaths indicates that 5 per cent. might have been saved if the treatment machinery in the community had at all times during the Demonstration been operating with complete success. It would seem, therefore, that so far as Framingham experience goes, a minimum of 16 per cent. would represent the figure that might be expected to die in a typical tuberculous group, including all kinds and stages of tuberculosis, after fairly complete treatment procedures, during a period of four years.

3. *The maximum number that may be reported before death.*

Framingham physicians, like physicians in other communities, at the beginning of the Demonstration were reporting from 60 to 80 per cent. of tuberculosis cases before death. During the last two years it has been possible, by encouraging reporting in all possible ways, to increase this figure to 93 per cent. Equally intensive work, over a longer period, might be expected to raise this percentage to more nearly the hundred maximum.

4. *The extent of tuberculosis in the population.*

Framingham was chosen for this work particularly because it was a representative American community, industrially, economically, socially, and racially, as regards sex and age distribution, etc.

A thorough examination of thousands of the population indicates a prevalence of active tuberculosis of about 1 per cent. Upon examination it is apparent that another 1 per cent. will have tuberculosis in an arrested stage.

Another basis for comparison is the ratio of known active cases to annual deaths in the community. The average normal American community is at the present time aware ordinarily of 3 to 5 known active cases for each annual death. At the beginning of the Demonstration the ratio in Framingham was 3 to 1. An intensive search with new machinery for discovering cases, during the first year, increased this ratio to 9 to 1.

These findings would indicate the importance of discovering tuberculosis, if the disease is to be brought under control. Incidentally, they also indicate the importance of adequate medical machinery for disease detection.

5. *The minimum number of cases needing sanatorium treatment.*

In Framingham at the beginning of the Demonstration, about 15 per cent. of the cases were receiving institutional care. For the first three years of the Demonstration this percentage was increased to 33, and in 1920 the figure was 24 per cent. This means that very nearly one third of the active cases in the community require institutional care, and can be materially benefitted by that form of treatment. This of course includes those cases which may be sent away for a few months, as well as those cases which may be away for several years. This practice would recognize the educational and hygienic advantages of even a short term under institutional instruction, as well as the direct therapeutic advantages of this type of treatment.

6. *The minimum number of beds required for tuberculosis treatment.*

Framingham, during the Demonstration, has been using from 1 to 2 beds in institutions for the treatment of active tuberculosis, in ratio to every annual death. Certainly a lower ratio than 1 bed to each annual death means an inadequate equipment, if thorough case finding work is carried out. A city of 100,000 people, therefore, with

100 deaths a year, would need at least 100 institutional beds for the treatment of pulmonary and non-pulmonary tuberculosis among adults and children.

7. *Minimum mortality rates.*

It is of course too early to discuss tuberculosis prevention in final terms. So far as the Framingham experience is concerned, little light has yet been thrown on the preventability of tuberculous infection. The repetition of a tuberculin survey at the end of the Demonstration, to contrast these observations with the findings at the beginning of the Demonstration in the original tuberculin survey, is expected to furnish data on this point. As to morbidity, the Demonstration is certainly now finding fewer cases, particularly fewer advanced cases. On mortality the experience is shown in the following figures:

	Per 100,000.
1907–1916	121.5
1916	78.3
1917	97.5
1918	84.7
1919	90.2
1917–1919	90.8
1920	64.5
1917–1920	84.2

It should be pointed out that these rates are corrected for non-residence, being based on residents dying in Framingham or out of town. They are also corrected for certification errors, based on a careful study of death certificates and concomitant field observations. The rates are also corrected for population re-estimated on the basis of the 1920 census.

It will be observed that the rate for the complete Demonstration period (1917–1920) when compared with the previous decade rate (1907–1916), represents a reduction of 31 per cent. or about one third. The rate for 1920 represents a reduction of 47 per cent., or about one half.

The Appraisal Committee, which studied the Framingham work about one and one half years ago, recommending its continuation beyond the original three-year period, made a careful study of the past tuberculosis mortality in Framingham, and worked out certain minimum and maximum normal variations that might be expected in a population of the size of Framingham (17,000), over a period of years. This committee stated that for a period of five years a rate which averaged below 90 per 100,000 would be significant as reflecting directly the special efforts to decrease tuberculosis. Of course the National Committee in charge of the Framingham work did not anticipate an exceptional showing as to mortality during the first two or three years of the Demonstration. It was rather expected that the rate might be increased owing to a greater interest on the part of the physicians in tuberculosis. a great increase in the detection of tuberculosis, in the reporting, and in the amount certified as a cause of death. However, if the rate for 1921 proves to be as low as that for 1920, the average rate for the five years of the Demonstration (1917–1921) will then be approximately 80 per 100,000, which is 10 points below the average set by the Appraisal Committee. Such a rate, while it would be considered significant and gratifying, ought in fact to be even further lowered, if the past experience and present tendencies in mortality are reasonably safe indications of future probabilities.

ANTIPNEUMOCOCCUS PROTECTIVE SUBSTANCES IN NORMAL CHICKEN SERUM.*

By CARROLL G. BULL, M. D., and CLARA M. McKEE.

(Received for publication, February 23d, 1921.)

The natural immunity of birds, particularly the pigeon and the domestic fowl, to infection by the pneumococcus has been a subject of research on the part of experimental immunologists since the earliest days of bacteriology. The solution of the mechanism of this phenomenon has been the chief objective, and the desire of the investigators has been to acquire a knowledge of the fundamental laws of immunity phenomena which might serve to guide them in the production and use of artificial immunity as a prophylactic and therapeutic agent.

While studying his "maladie nouvelle" (septicemia produced in rabbits by inoculations of sputum) Pasteur (1) discovered a new organism which, on microscopic examination, reminded him of the bacillus of chicken cholera. He inoculated chickens with the new organism and found them to be quite refractory to it. After the identity of Pasteur's sputum-septicemia organism and the pneumococcus of Fränkel was established, other workers became interested in Pasteur's original observation and it was soon found that not only chickens but pigeons and other birds are highly resistant to infection by the pneumococcus. Fränkel (2), Gamaléia (3), Tchistovitch (4) and Welch (5) were among the earliest workers who confirmed and extended Pasteur's experiments. More recently Strouse (6), Keyes (7) and Berry and Melick (8) have done interesting and important work on this subject, especially on the mechanism of the phenomenon.

Tchistovitch (4) concluded that wandering phagocytic cells play a decisive part in the pigeon's resistance to the pneumococcus. This author injected pneumococcus cultures into the anterior eye chamber of pigeons and found that the organisms did not multiply and that phagocytes soon appeared and engulfed the cocci which he had injected. Similar experiments performed on the rabbit showed that in this animal the cocci rapidly increased in number, few phagocytes appeared and a general infection developed causing death within a short time. Strouse's (6) experiments seemed to show that the normal high temperature of the pigeon is an important, if not decisive, factor in its immunity to the pneumococcus. Keyes (7) questions Strouse's conclusion and presents experiments which show that certain phagocytic cells (the hemophages) of the liver and spleen are very active in the destruction of pneumococci following intraperitoneal or intravenous injections of the organism. Keyes admits the possibility of a high temperature influence but the results of his experiments on this point tend to exclude the temperature of the pigeon as an important factor.

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That phagocytic cells finally take up and destroy pneumococci which have been injected into refractory animals (including both naturally immune and artifically immunized animals) seems to be well established. It is also well established that the sera of immunized animals contain immune bodies which can be demonstrated in vitro, and also confer passive immunity upon susceptible animals. Whether normal humoral factors play a significant or esential part in the mechanism of the resistance of naturally immune animals is, however, still debatable. Neither Strouse (6) nor Keyes (7, 9) found any evidence of antibodies in the body fluids of either the chicken or the pigeon. In testing the serum of chickens super-immunized with pneumococcus, Keyes (9) used the serum of normal chickens as a control and found that the normal serum did not protect mice against infection with the pneumococcus, while the serum of the artificially immunized chickens was very potent in this respect. Bull and Bartual (10) published experiments which showed that pneumococci are phagocyted in the fresh, undiluted blood of both chickens and pigeons. This furnished at least presumptive evidence that the blood contained opsonins for the pneumococcus since it is a general rule that washed cells of the most resistant animal are not capable of phagocyting virulent bacteria (pneumococci particularly) if they have not been previously sensitized (opsonized) with the serum of an immune animal. In a further investigation of the humoral phase of the natural immunity of the domestic fowl to pneumococci, we have made the observations presented in this paper.

EXPERIMENTAL.

Our first experimental objective was to prove the presence or absence of protective substances in normal chicken serum for any or all of the types of pneumococci. Experiments were planned with the view of affording optimal conditions for protection. Experiment A will serve to illustrate the type of protocol used for this purpose.

Experiment A.—(*a*) The serum was obtained by taking blood (20 to 40 cc.) from each of four or five large hens or roosters. The blood was collected aseptically, allowed to clot at room temperature, put in an ice box over night and the clear serum removed the next morning.

(*b*) The strains of pneumococci used in this and subsequent experiments had been passed through mice and possessed a high degree of virulence for these animals, the lethal dose being not less than 0.0000001 cc. of an eighteen-hour culture. The cultures were grown in veal infusion broth to which defibrinated rabbit blood (5 per cent. by volume) had been added.

(*c*) Mice of about 25 grams weight were given, intraperitoneally, 1 cc. of fresh chicken serum and four hours later inoculated, also intraperitoneally, with from 10 to 100,000 lethal doses of pneumococcus culture. Control mice which had received no serum were infected at the time of inoculating the test animals.

The experiment is tabulated in Protocols 1, 2, 3, 4, 5 and 6 which represent respectively infection with pneumococci of Types I, II, II A, II B, III and Group IV.

PROTOCOL 1.

Mouse protection against Type I pneumococcus with normal chicken serum.

Mice.	Serum.	Type II pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 36 hrs.

D., dead; S., survived 5 days and discarded.

PROTOCOL 2.

Mouse protection against Type II pneumococcus with normal chicken serum.

Mice.	Serum.	Type I pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 24 hrs.

PROTOCOL 3.

Mouse protection against Type II A pneumococcus with normal chicken serum.

Mice.	Serum.	Type II A pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 36 hrs.

PROTOCOL 4.

Mouse protection against Type II B pneumococcus with normal chicken serum.

Mice.	Serum.	Type II B pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 36 hrs.

PROTOCOL 5.

Mouse protection against Type III pneumococcus with normal chicken serum.

Mice.	Serum.	Type III pneumococcus.	Results.
1......	1 cc.	0.0001 cc.	D. 48 hrs.
2......	1 cc.	0.00001 cc.	S. 5 days
3......	1 cc.	0.000001 cc.	S. 5 days
4......	1 cc.	0.0000001 cc.	S. 5 days
5......	—	0.0000001 cc.	D. 36 hrs.

PROTOCOL 6.

Mouse protection against Group IV pneumococcus with normal chicken serum.

Mice.	Serum.	Group IV pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 36 hrs.

The protocols above, which were repeated a number of times, show very decisively that the serum of normal chickens contains protective substances for every type of the pneumococcus. The serum gave less protection against Type III than against the other types. In no instance did mice survive which had received more than 0.00001 cc. of culture. The 24-hour lethal dose of this culture was 0.0000001 cc.

Protection against the other types was quite regular up to 0.001 cc. of culture. Occasionally mice which had received 0.001 cc. died of infection from two to five days after being inoculated and there were sometimes irregular deaths, i.e., a mouse which had received 0.0001 cc. of culture would die while the mouse having received ten times this amount of culture survived. The most regular results were obtained in protection against our Type II culture. Time was saved by always running duplicate experiments and thus eliminating the disturbance of irregular deaths from specific infection and accident.

Experiments planned to determine the optimum conditions for protection and the nature of the protective substance gave the following results: (1) Mice manifest the highest degree of resistance when infected from eight to twelve hours after the serum has been given; there is much less protection when the serum and culture are mixed and immediately injected; twenty-four hours after the administration of the serum the mice are only slightly more resistant than normal controls. (2) If the serum is given subcutaneously, the mice do not manifest an appreciable increased resistance at any time. (3) Less than 0.5 cc. of the serum affords little protection; while 3 cc. of serum is injurious to the mice. Two cc. of serum gives more protection than 1 cc. but the difference is not great. (4) Serum from 2 to 3 weeks old is as potent as fresh serum and it is less toxic. (5) Heating the serum for thirty minutes at 56° C. does not affect its potency, while serum heated for thirty minutes at 65° C. has lost its protective power. (6) The serum of old chickens is more potent than that of young ones.

Two other points that have been determined are of sufficient interest to be given in detail: (1) The fraction of the serum in which the protective substances reside, and (2) the type specificity of the protective substances.

Fractionation of the serum.

For this work the serum was obtained by collecting blood from a large number (100 to 200) of chickens, allowing it to clot, and centrifugalizing the clot within two or three hours after collecting the blood. The serum was not sterile but there was no opportunity for bacterial growth. A type experiment is given below (Experiment B).

Experiment B.—Two liters of serum were diluted with four liters of tap water. Two liters of a saturated solution of ammonium sulphate were slowly added to the diluted serum, the mixture being constantly stirred. This stood at room temperature until the following day. A light, flocculent precipitate (precipitate A) had formed. The precipitate was collected on hardened filter paper. 3.75 liters of saturated sulphate solution were added to 7.5 liters of the filtrate obtained above. This caused a heavy precipitate to form which was filtered off the next day. This precipitate will be designated precipitate B. The filtrate obtained here was saturated with ammonium sulphate crystals and allowed to stand for 24 hours when the precipitate, which was now rising and leaving a clear and colorless fluid below, was collected on hardened filter paper. This last precipitate will be called precipitate C. The three precipitates were thoroughly pressed and then put into collodion bags and dialyzed in running tap water until they were free of the sulphate. The contents of the bags containing precipitates A and C were removed and rendered isotonic with sodium chloride cystals. Precipitate C went into solution during the dialysis and did not reprecipitate as the sulphate disappeared. Precipitate A did reprecipitate and dissolved only slightly on the addition of the sodium chloride, but almost complete solution was effected by rendering it alkaline with sodium bicarbonate. A heavy precipitate had formed in the bag containing precipitate B. A portion of the supernatant fluid was removed and rendered isotonic with sodium chloride. The precipitate and the remainder of the supernatent fluid were removed together and made isotonic. The precipitate dissolved immediately on the addition of the salt. The four solutions obtained from the dialysates were passed through Mandler filters and then tested for antipneumococcus protective substances. The first solution (''fibrinoglobulin''), it will be remembered, came from precipitate A which was formed when one fourth volume of saturated ammonium sulphate was added to the diluted serum. The second solution (''pseudoglobulin'') is the supernatant fluid from the dialysate of precipitate B (one half saturation precipitate) and is therefore largely the water soluble globulin. The third solution (''euglobulin'') is from the precipitate from dialysate B and consists principally of the water insoluble globulin. The last solution (albumin) is the dialysate from precipitate C or the precipitate which formed between 50 and 100 per cent. saturation of the serum with ammonium sulphate and consists, therefore, of the albumin fraction of the serum.

Guinea pigs weighing between 180 and 200 grams were used in testing the potency of the serum fractions which we have just described. A strain of Type II pneumococcus was used to infect the pigs. This strain had been passed through a number of guinea pigs and was very virulent for these animals, 0.000001 cc. of an 18-hour blood broth culture being the 24-hour lethal dose. Mice were also used in a few experiments and the results corresponded with those obtained with the guinea pigs. Protocols 7, 8, 9, and 10 will serve to illustrate the type of experiment employed in these tests and the results obtained. The guinea pigs were infected three hours after the serum fractions had been given, both being given intraperitoneally.

PROTOCOL 7.

Guinea pig protection against Type II pneumonococcus with the "Fibrinoglobulin" of chicken serum.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	2 cc.	0.01 cc.	D. 40 hrs.
2......	2 cc.	0.001 cc.	D. 4 days
3......	2 cc.	0.0001 cc.	D. 3 days
4......	2 cc.	0.00001 cc.	D. 4 days
5......	—	0.000001 cc.	D. 40 hrs.
6......	2 cc.	—	S. 7 days

D., dead; S., survived 7 days and discarded.

PROTOCOL 8.

Guinea pig protection against Type II pneumococcus with the "Pseudoglobulin" of chicken serum.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	2 cc.	0.01 cc.	S. 7 days
2......	1 cc.	0.01 cc.	D. 6 days
3......	1 cc.	0.001 cc.	S. 7 days
4......	1 cc.	0.0001 cc.	S. 7 days
5......	—	0.000001 cc.	D. 40 hrs.
6......	2 cc.	—	S. 7 days

PROTOCOL 9.

Guinea pig protection against Type II pneumococcus with the "Euglobulin" of chicken serum.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	2 cc.	0.1 cc.	S. 7 days
2......	1 cc.	0.1 cc.	D. 6 days
3......	1 cc.	0.01 cc.	S. 7 days
4......	1 cc.	0.001 cc.	S. 7 days
5......	—	0.000001 cc.	D. 40 hrs.
6......	2 cc.	—	S. 7 days

PROTOCOL 10.

Guinea pig protection against Type II pneumococcus with the albumin of chicken serum.

Guinea pig.	Albumin.	Type II pneumococcus.	Results.
1......	2 cc.	0.1 cc.	D. 21 hrs.
2......	2 cc.	0.01 cc.	D. 24 hrs.
3......	2 cc.	0.001 cc.	D. 28 hrs.
4......	2 cc.	0.0001 cc.	D. 40 hrs.
5......	—	0.000001 cc.	D. 40 hrs.
6......	2 cc.	—	S. 7 days

From Protocol 7 it is seen that the precipitate formed on adding one fourth volume of saturated suluphate solution to the diluted serum contained very little of the protective substances. Two cc. of the dialysate from this precipitate did not give permanent protection. Other tests showed that the precipitate formed by 23 per cent. saturation did not contain even a trace of the protective substances. The albumin fraction did not give protection (Protocol 10). Both the water-soluble and the water-insoluble fractions gave permanent protection (Protocols 8 and 9). The water-insoluble fraction was, however, definitely more potent than the water-soluble fraction, 2 cc. of the former protecting against 0.1 cc. of culture and 1 cc. against 0.01 cc. of culture, while it took 2 cc. of the water-soluble fraction to protect against 0.01 cc. of culture. These results might be taken to indicate that the protective substances are associated with both globulin fractions but the methods used in this experiment did not insure complete separation of the two fractions. A further experiment (Experiment C) was undertaken in which a complete separation of the globulin on the basis of water solubility was attempted.

Experiment C.—The globulin was removed from the serum by salting and consisted of the precipitate which formed on one half saturation with ammonium sulphate, the one fourth saturation precipitate having been previously removed. The precipitate was dialyzed against running tap water until it was free of sulphate. The fluid portion of the dialysate was removed and dialyzed against distilled water for 24 hours. A precipitate had formed. This was removed by filtering through hardened paper and the filtrate was put into a new bag and redialyzed. This process was repeated until a 24-hours' dialysis against distilled water caused no precipitate to form. The dialysate was now rendered isotonic filtered and tested for antipneumococcus protective potency. The solid portion of the original dialysate was put into two liters of distilled water, thoroughly shaken and recollected by centrifugalization. The washing process was repeated and the sediment from the centrifugalization was dissolved in the smallest possible quantity of physiological salt solution. This solution was filtered and tested for antipneumococcus protective power. The protection tests are given in Protocols 11 and 12.

PROTOCOL 11.

Guinea pig protection against Type II pneumococcus with water-insoluble globulin.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	0.1 cc.	0.1 cc.	D. 40 hrs.
2......	0.1 cc.	0.01 cc.	S. 7 days
3......	0.1 cc.	0.001 cc.	S. 7 days
4......	0.2 cc.	0.1 cc.	S. 7 days
5......	0.2 cc.	0.01 cc.	S. 7 days
6......	0.2 cc.	0.001 cc.	S. 7 days
7......	—	0.000001 cc.	D. 40 hrs.

PROTOCOL 12.

Guinea pig protection against Type II pneumococcus with water-soluble globulin.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	2.4 cc.	0.1 cc.	D. 40 hrs.
2......	2.4 cc.	0.01 cc.	D. 40 hrs.
3......	2.4 cc.	0.001 cc.	D. 40 hrs.
4......	—	0.000001 cc.	D. 40 hrs.
5......	2.4 cc.	—	S. 7 days

Protocols 11 and 12 show that only the water-insoluble fraction of the globulin contains the protective substances. Two tenth of this fraction of the serum protected against 0.1 cc. of culture or 100,000 lethal doses, while 2.4 cc. of the water-soluble fraction did not protect against 0.001 cc. of culture.

TYPE SPECIFICITY OF THE PROTECTIVE SUBSTANCES.

It has been shown above that the serum of chickens protects mice and guinea pigs against any of the various types of pneumococci. Since in artificially produced immune sera the protective substances are type specific (a distinct substance for each type) and can be selectively removed by bacterial adsorption (11), it becomes of interest to know whether the chicken serum contains a distinct protective substance for each type or only one substance which has the property of protecting against any and all of the different types. The principle of selective antibody adsorption was here taken advantage of. The details of the work are given under Experiment D.

Experiment D.—(General technique.) About 150 cc. of a 24-hour broth culture of pneumococci was centrifugalized at high speed until the cocci were packed firmly on the bottom of the tubes. The supernatant culture fluid was carefully and completely removed, an effort being made to get the bacteria as dry as possible. Fresh chicken serum (30 cc. for each 150 cc. of culture) was put into the tubes containing the sedimented culture and agitated until an even suspension of the cocci had been effected. The suspension was placed in the incubator (37° C.) for two hours and then sedimented by centrifugalization. The supernatant serum was removed and passed through a Mandler filter to remove the remaining bacteria. A series of three mice for each type of pneumococcus to be used for infection was given, intraperitoneally, 1 cc. each of the adsorbed serum, and an equal number of mice was given 1 cc. of an unadsorbed portion of the same lot of serum. Four hours later all the mice were infected as indicated in the following representative protocols.

PROTOCOL 13*a*.

Mouse protection against Types I and II pneumococcus with chicken serum adsorbed with Type I pneumococcus.

Mice.	Ad. S.	N. S.	Pn. I.	Pn. II.	Results.
1....	1 cc.		0.0001 cc.		D. 24 hrs.
2....	1 cc.		0.00001 cc.		D. 24 hrs.
3....	1 cc.		0.000001 cc.		D. 30 hrs.
4....		1 cc.	0.0001 cc.		S. 5 days
5....		1 cc.	0.00001 cc.		S. 5 days
6....		1 cc.	0.000001 cc.		S. 5 days
7....	1 cc.			0.0001 cc.	S. 5 days
8....	1 cc.			0.00001 cc.	S. 5 days
9....	1 cc.			0.000001 cc.	S. 5 days
10....		1 cc.		0.0001 cc.	S. 5 days
11....		1 cc.		0.00001 cc.	S. 5 days
12....		1 cc.		0.000001 cc.	S. 5 days
13....			0.0000001 cc.		D. 30 hrs.
14....				0.0000001 cc.	D. 24 hrs.

Ad. S., Adsorbed serum.

N. S., Normal serum.

Pn. I, 18-hour blood broth culture of Type I pneumococcus.

Pn. II, 18-hour blood broth culture of Type II pneumococcus.

PROTOCOL 13*b*.

Mouse protection against Types I and II pneumococcus with chicken serum adsorbed with Type II pneumococcus.

Mice.	Ad. S.	N. S.	Pn. I.	Pn. II.	Results.
1....	1 cc.		0.0001 cc.		S. 5 days
2....	1 c.c		0.00001 cc.		S. 5 days
3....	1 cc.		0.000001 cc.		S. 5 days
4....		1 cc.	0.0001 cc.		S. 5 days
5....		1 cc.	0.00001 cc.		S. 5 days
6....		1 cc.	0.000001 cc.		S. 5 days
7....	1 cc.			0.0001 cc.	D. 24 hrs.
8....	1 cc.			0.00001 cc.	D. 24 hrs.
9....	1 cc.			0.000001 cc.	D. 24 hrs.
10....		1 cc.		0.0001 cc.	S. 5 days
11....		1 cc.		0.00001 cc.	S. 5 days
12....		1 cc.		0.000001 cc.	S. 5 days
13....			0.0000001 cc.		D. 36 hrs.
14....				0.0000001 cc.	D. 24 hrs.

From Protocols 13 *a* and 13 *b* it is seen that serum adsorbed with Type I pneumococci no longer protects mice against this type but still protects against Type II as well as the unadsorbed serum, and that adsorption with Type II removes the protective substance for this type without removing the Type I protective substance. It appears, therefore, that normal chicken serum contains distinct protective substances for these two types of pneumococci.

Other experiments, similar to Experiment D, showed that chicken serum contains a specific protective substance for each type of pneumococcus. Only one virulent strain of Group IV was available but serum adsorbed with this strain no longer protected against itself but still protected against Types I, II and III, and adsorption with any of these types did not remove the protective substance for the Group IV strain.

It now became of interest to know whether the type specificity of chicken serum extended to the subtypes of Type II strains. These subtypes, it will be remembered, consist of atypical Type II organisms or strains of pneumococci which are agglutinated only slowly and incompletely with Type II serum. In a study of these atypical Type II strains Avery (11) discovered two new serological types which he designated Type II A and Type II B. The relation between these new types and Type II was as follows: A Type II immune serum contains agglutinins and protective substances for all three types but an immune serum produced with a strain of either of the other types (II A or II B) contains antibodies only active against the homologous type. A Type II serum adsorbed with a Type II culture no longer protects against or agglutinates Type II, II A or II B strains, all the antibodies being removed. If a Type II serum is adsorbed with either II A or II B organisms only the homologous antibodies are removed, and the antibodies are not removed from a Type II A immune by adsorption with a II B culture and vice versa. It is seen, therefore, that a Type II immune serum contains one group of major antibodies and two groups of minor antibodies, the latter groups being distinct from each other or, in other words, Types II A and II B are subgroups of Type II.

Avery's experiments were repeated, using chicken serum instead of the sera of immunized animals. Our work is given in detail under Experiment E.

*Experiment E.**—Three series of mice were given, intraperitoneally, 1 cc. of chicken serum which had been adsorbed (see Experiment D) with either a Type II, II A or II B culture and were infected four hours later—one series with Type II culture, one with Type II A and one with Type II B. The three following protocols give the different experiments in tabular form.

PROTOCOL 14.

Mouse protection against Types II, II A and II B pneumococcus with chicken serum adsorbed with Type II pneumococcus.

Mice.	Ad. S.*	Pn. II.	Pn. II A.	Pn. II A.	Results.
1....	1 cc.	0.0001 cc.			D. 24 hrs.
2....	1 cc.	0.00001 cc.			D. 24 hrs.
3....	1 cc.	0.000001 cc.			D. 24 hrs.
4....	1 cc.		0.0001 cc.		D. 3 days
5....	1 cc.		0.00001 cc.		S. 5 days
6....	1 cc.		0.000001 cc.		S. 5 days
7....	1 cc.			0.0001 cc.	D. 2 days
8....	1 cc.			0.00001 cc.	S. 5 days
9....	1 cc.			0.000001 cc.	S. 5 days
10....		0.0000001 cc.			D. 24 hrs.
11....			0.0000001 cc.		D. 48 hrs.
12....				0.0000001 cc.	D. 48 hrs.

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* The strains of Types II A and II B pneumococci used in this experiment were kindly furnished us by Dr. O. T. Avery, of the Hospital of the Rockefeller Institute.

* The same number of mice was given 1 cc. each of an unadsorbed portion of the same lot of serum and infected in the same way and at the same time as the

PROTOCOL 15.

Mouse protection against Types II, II A and II B pneumococcus with chicken serum adsorbed with Type II A pneumococcus.

Mice.	Ad. S.	Pn. II.	Pn. II A.	Pn. II B.	Results.
1....	1 cc.	0.0001 cc.			D. 24 hrs.
2....	1 cc.	0.00001 cc.			D. 24 hrs.
3....	1 cc.	0.000001 cc.			D. 24 hrs.
4....	1 cc.		0.0001 cc.		D. 24 hrs.
5....	1 cc.		0.00001 cc.		D. 24 hrs.
6....	1 cc.		0.000001 cc.		D. 48 hrs.
7....	1 cc.			0.0001 cc.	S. 5 days
8....	1 cc.			0.00001 cc.	S. 5 days
9....	1 cc.			0.000001 cc.	S. 5 days
10....		0.0000001 cc.			D. 24 hrs.
11....			0.0000001 cc.		D. 48 hrs.
12....				0.0000001 cc.	D. 48 hrs.

PROTOCOL 16.

Mouse protection against Types II, II A and II B pneumococcus with chicken serum adsorbed with Type II B pneumococcus.

Mice.	Ad. S.	Pn. II.	Pn. II A.	Pn. II B.	Results.
1....	1 cc.	0.0001 cc.			D. 24 hrs.
2....	1 cc.	0.00001 cc.			D. 24 hrs.
3....	1 cc.	0.000001 cc.			D. 24 hrs.
4....	1 cc.		0.0001 cc.		S. 5 days
5....	1 cc.		0.00001 cc.		S. 5 days
6....	1 cc.		0.000001 cc.		S. 5 days
7....	1 cc.			0.0001 cc.	D. 24 hrs.
8....	1 cc.			0.00001 cc.	D. 24 hrs.
9....	1 cc.			0.000001 cc.	D. 48 hrs.
10....		0.0000001 cc.			D. 24 hrs.
11....			0.0000001 cc.		D. 48 hrs.
12....				0.0000001 cc.	D. 48 hrs.

The protocols above show, (1) that chicken serum adsorbed with Type II pneumococci loses only the Type II protective substances, still protecting against Type II A and II B strains; (2) that serum adsorbed with Type II A culture looses the Type II A and Type II antibodies but not the Type II B antibodies; and (3) that serum adsorbed with Type II B culture looses the Type II B and Type II protective substances but not those for Type II A.

mice which received the adsorbed serum in order to make sure that the serum contained protective substances for all three types before it was adsorbed. The same was done in Protocols 15 and 16.

In terms of the protective substances of chicken serum, therefore, Types II A and II B pneumococci seem to form two distinct main groups with Type II a subgroup to both of them. This relationship is just the reverse of that which Avery found in terms of sera from specifically immunized animals.

Discussion.

The observations recorded above, in conjunction with previous observations, afford ground for concluding that specific humoral elements are essential factors of the natural immunity of the chicken to infection by the pneumococcus. The exact nature of these humoral elements or the manner in which they operate to effect resistance has not been determined. It is probable, however, that they are of the nature of opsonins and prepare the pneumococci for phagocytic destruction. The basis of these conclusions is briefly as follows: (1) The serum of chickens has the power of conferring upon other animals an immunity to the pneumococcus, (2) virulent pneumococci are phagocyted in fresh chicken blood in vitro, (3) pneumococci grow readily in fresh chicken serum or plasma, and (4) opsonization and phagocytosis are known to be essential factors in other instances of acquired and artificial immunity to the pneumococcus.

It may be objected that the passive immunity conferred upon other animals with chicken serum is not comparable in degree to that of the chicken and that the methods employed fail to demonstrate pneumococcus opsonins in chicken serum. It is to be remembered, on the one hand, that it is not necessary to have a high concentration of antibodies in the body tissues and fluids of an animal in order to have protection against infection, provided the antibodies are constantly present and are inexhaustible. On the other hand, the usual methods of studying antibodies in vitro entail a dilution of the serum and a one-fold dilution may suffice to inhibit their action. These conditions seem to obtain in the present case. The production of antipneumococcus protective substances is, no doubt, a natural function of the tissues of the chicken and whenever the normal concentration of these substances in the blood is reduced the tissues immediately liberate more of them and reestablish the equilibrium, the resistance of the animal being thus maintained. As already stated, pneumococci are phagocyted in the undiluted fresh blood of the chicken while in a mixture of one part serum, one part bacteria and one part leucocytes no phagocytosis occurs. The demonstration by Keyes (7) that phagocytosis plays a prominent rôle in the destruction of pneumococci in the pigeon is another point in favor of this explanation of the chicken's immunity to the pneumococcus. In all probability similar mechanisms operate in both instances, but as yet the humoral phase in the former and phagocytic in the latter have not been satisfactorily demonstrated.

Why chicken serum protects mice against infection with the pneumococcus when it is given intraperitoneally and fails to protect when it is given subcutaneously is not entirely clear. There are two facts, however, which afford a possible explanation for this anomaly. First, animals are more resistant to infection several hours after they have received antibacterial sera than they are if they are infected at the time of administering the serum. This is true notwithstanding the fact that the infecting organisms may be exposed to the action of the concentrated serum when the culture and the serum are given at the same time and not when the serum is given several hours before the animals are infected. It appears, therefore, that some kind of reaction between the immune serum and the tissues of the host must take place before the protective serum becomes effective. This reaction may be nothing more than the adsorption of the protective bodies by the cells of the host or it may be a more specific reaction between factors from the host and from the immune serum resulting in the formation of a new substance to which protection is due. In any case, time elapses between the administration of the serum and the development of the maximum resistance on the part of the host. The second fact is that the chicken serum is rapidly destroyed or eliminated by mice for, on intraperitoneal injection, the mice are not protected for more than twenty-four hours. Horse serum is not so quickly eliminated since antipneumococcis horse serum protects mice for at least six days. It is possible, therefore, that on subcutaneous injection the chicken serum is destroyed before the reaction necessary for protection occurs and, for this reason, a resistant state never develops.

The discovery of antibodies in the serum of a normal animal which are specific for the different serological types of the pneumococcus shows that the classification of these organisms, although done with artificially prepared sera, has a fundamental biological significance. If this classification had first been done by means of the protective substances of chicken serum, it would have been just as it is with the exception of the relation of the group II subtypes to the main type. This difference is due, doubtless, to differences in the antigenic origin of the two sets of antibodies. The observation is of interest in connection with existing theories of the origin and relationships of major and minor antibodies.

How the chicken came to possess these antipneumococcic substances is quite a puzzle to the immunologist. Existing theories concerning the origin of natural bacterial antibodies do not offer a satisfactory explanation in this instance. Is it possible that these substances owe their origin to a specific antigenic stimulation which occurred in some earlier period of the life history of the species, the germplasm being so fundamentally impressed that an acquired property became inheritable? If specific antigenic stimulation is eliminated, it gives reason for questioning the soundness of this theory with reference to other natural antibodies found in both man and the lower animals.

SUMMARY.

1. It has been shown that the serum of normal chickens is capable of protecting mice and guinea pigs against infection with pneumococci.
2. The protective substances are found in the water-insoluble fraction of the serum globulin.
3. There are, in chicken serum, particular protective substances for each serological type of pneumococcus. These substances are selectively removed from the serum by the process of bacterial adsorption.
4. In terms of the protective substances in chicken serum, Types II A and II B pneumococci constitute two distinct main groups and Type II strains form a subgroup to both of them.

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THE SIGNIFICANCE OF ALBUMIN AND OF ALBUMIN WITH CASTS IN THE URINE.*

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The following study was undertaken to determine, if possible, the importance of persistent albumin and of albumin with casts as a physical defect measured in terms of mortality. It is based upon the after-life history of about 5,200 persons who were rejected for insurance in the Metropolitan Life Insurance Company in the period between 1905 and 1915. So far as it was possible, the study was limited to cases in which there were no other serious complications which are often found in connection with albuminuria, such as, heart affections, a history of renal disease or of renal colic. As these were rejected lives, it was anticipated that they would show higher death rates during the follow-up period than unimpaired lives at comparable ages; but, every precaution had to be taken to define the group so as to include within it those who had only the single impairment, namely, persistent albumin or albumin with casts. The excess mortality actually found to prevail in each group would, therefore, measure the significance of the impairment.

The method followed was to trace the whereabouts of these rejected persons since the period of the examinations. Some had previous insurance in this and in other companies. It was comparatively easy to follow them through this channel. Those that had no other insurance records were traced by means of local correspondents who made careful investigation of the present whereabouts of these persons. Every known safeguard was taken to locate persons now living and to record the deaths which had occurred; and, in the latter group, to obtain the date and the cause of death. There is every indication that our findings present a fair picture of the actual history of these people during the period subsequent to their rejection by the insurance company.

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* The writer acknowledges his indebtedness to Dr. J. Bergen Ogden and to Dr. A. W. Balch, Assistant Medical Directors of the Metropolitan Life Insurance Company for their valuable assistance in planning this study and for their criticism and suggestions during its progress.

(*a*) Cases with albumin only.

The first group studied consisted of 2,073 persons who showed albumin on several examinations in amounts exceeding the slightest possible trace. No casts were found on microscopic examination. These persons were followed up from the date of their rejection up to December 31, 1918. The entire group was under observation for an aggregate period of 12,078 years and the average period of observation was close to six years since their insurance examination. A total of 161 deaths occurred among them during this period. This is the actual mortality. In comparison with these actual deaths, we have calculated the number of deaths that might have been expected to occur if the death rates for each age period were in accordance with certain standard tables. The first of these is that of Professor Glover* for white males living in the Registration Cities of the United States in 1910. It reflects the conditions in the general population and includes everybody, sick and well alike. According to this table, 141 deaths should have occurred among these people. The actual deaths were, therefore, 114 per cent. of the expected according to the table. The best criterion, however, for our purpose is the very recent American Men Table.† This reflects the actual experience among the best type of insured lives in recent years in American companies. On this table, only 88 deaths would have occurred as against 161. The actual mortality was, therefore, 183 per cent. of that which might have been expected in a group of insured people at the same ages who were presumably free from serious defects. This per cent. of excess mortality, we believe, measures the significance of the urinary impairment.

The following table shows the above facts of mortality by age period:

It would appear, therefore, that the persistent presence of albumin in quantities above the slightest possible trace is a serious impairment. The total mortality is not far from double that for the best grade of insured persons even during the relatively short period, from 3 to 13 years, following the original examinations. The excessive

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* United States Life Tables, 1910, Bureau of the Census, 1916, pp. 38–39.

† American-Canadian Mortality Investigation, 1918—Mortality Experience on Insured American Men, p. 53.

TABLE I.

Mortality among rejected applicants showing albumin—all quantities. Exposures, deaths, and death rates per 1,000. Per cent. actual to expected mortality by various tables.

Attained age.	Exposure years.	Actual deaths.	Death rate per 1,000.	Per cent. actual to expected deaths.	
				Glover.	American Men.
All ages.......	12,077.88	161	13.33	114	183
15 to 24.......	2,134.58	12	5.62	125	145
25 to 34.......	4,490.84	37	8.24	114	184
35 to 44.......	3,067.73	48	15.65	130	267
45 to 54.......	1,661.65	37	22.27	116	194
55 to 64.......	618.32	23	37.20	103	142
65 to 74.......	104.76	4	38.18	52	63

mortality is manifest at virtually every attained age period, but is most marked at the ages 35 to 44 when the actual deaths are 267 per cent. of the expected mortality according to the American Men Table, or nearly three times as high as the expected. The excess in the mortality drops after this age period and disappears entirely for the ages 65 to 74, but the exposures are too few to permit any conclusions to be drawn from the ratio.

The relatively high mortality (145 per cent. of the expected, according to the A. M. Table) at the ages 15 to 24, is especially instructive in view of the recent findings by many writers both in the United States and in England that albuminuria in adolescents is generally of no significance. Our high figure suggests further analysis. As we shall see more fully later, the high mortality at these ages, 15 to 24, is limited entirely to those persons who have a trace or more of albumin. Those with a faint trace of albumin appear to have a mortality rate even better than the expected, which is not inconsistent with recent clinical opinion. It is very important to distinguish in these albuminurias among very young people between those that have very small and those that have larger quantities of albumin.

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* The Ogden scale was followed in this investigation. See ''Clinical Examination of the Urine,'' by J. Bergen Ogden, pp. 125 to 127, W. B. Saunders Co., Philadelphia-London, 1909.

1. Faint trace of albumin includes slightest possible trace and very slight trace in the Ogden scale.

2. Trace of albumin includes slight trace and trace.

3. Moderate or large quantity of albumin includes large trace or over.

It is necessary, therefore, to consider the mortality findings according to the amount of albumin. Three sub-classes* were distinguished, namely, those with (1) a faint trace of albumin; (2) a trace of albumin; and (3) a moderate or large quantity of albumin. Unfortunately, the last of the three included too few persons to permit analysis. An interesting comparison can be made, however, of the first two. Table II presents the facts for this comparison with distinction for attained age period.

TABLE II.

Mortality among rejected applicants showing "faint trace" and "trace" of albumin. Exposures, deaths and death rates per 1,000 by attained ages. Per cent. actual to expected deaths by various tables.

Attained age.	Faint trace of albumin.					Trace of albumin.				
	Exposure years.	Actual deaths.	Death rate per 1,000.	Per cent. actual to expected deaths. Glover.	Per cent. actual to expected deaths. American Men.	Exposure years.	Actual deaths.	Death rate per 1,000.	Per cent. actual to expected deaths. Glover.	Per cent. actual to expected deaths. American Men.
All ages.......	6,762.08	91	13.46	105	169	4,426.54	62	14.01	139	227
15 to 24......	1,006.00	3	2.98	59	73	924.99	8	8.65	174	216
25 to 34......	2,332.25	19	8.15	113	181	1,807.75	17	9.40	132	210
35 to 44.......	1,817.58	28	15.41	129	264	1,046.48	15	14.33	122	246
45 to 54.......	1,066.08	22	20.64	109	185	491.42	15	30.52	163	278
55 to 64......	442.91	15	33.87	91	132	148.40	7	47.17	135	194
65 to 74......	97.26	4	41.13	60	75	7.50	0	—	—	—

There is a marked difference in the amount of extra mortality in the two groups. As measured by the A. M. Table, those having a faint trace of albumin gave a mortality rate of 169 per cent. of the expected for all ages combined; whereas those with a trace of albumin, 227 per cent. of the expected. The age element, as we have already seen, also plays an important part in determining the differences in mortality. The most striking difference is in the age period 15 to 24, when those with a faint trace of albumin show no extra mortality, while those with a trace show more than a double mortality. Apparently, it is not an impairment for young people 15 to 24 years old to have a faint trace of albumin even persistently; when the amount of albumin rises to a trace, it appears to be a serious matter. Between 25 and 44 years, there is no marked difference between the two classes, although both show very excessive mortality from a little under two to more than two and one half times the expected. After age 45, those showing a faint trace of albumin suffer less seriously than those with a trace, but the findings are based on small numbers of people and may have no great value.

The increased mortality of the whole group showing albumin is largely due to the very high death rate from a few diseases and conditions. Bright's disease is especially in excess and so are also heart disease and tuberculosis of the lungs. The death rate for Bright's disease reached 306.3 per 100,000, as compared with a rate a little under 40 in the Ordinary experience of the company during recent years. As might be expected from our previous discussion, this disease has a very much higher incidence in the cases with larger amounts of albumin than in those showing only a faint trace of albumin, although even in the latter group, the death rate was 222 per 100,000, or over five times as high as in the Ordinary experience of the company. The highest mortality from Bright's disease prevailed among the older persons. Those who showed a faint trace of albumin over age 40 at the time of examination, had a death rate of about 450 per 100,000 from this disease and those with a trace of albumin had the astounding death rate of 1,262 per 100,000. The death rate from organic heart disease in the entire group was 74.5 per 100,000, which is 50 per cent. higher than the company's Ordinary experience of recent years. The rate for tuberculosis of the lungs is also excessive. The total death rate was 248.4 per 100,000, or about three times as high as in the Ordinary insurance experience. The tuberculosis rate is high for all these cases; but is strikingly so in the group 15 to 24 years old and showing a trace of albumin. Out of eight deaths, five were from tuberculosis, the rate for this disease reaching the figure of 541 per 100,000. There is obviously a very close connection between albuminuria and an excessive mortality from tuberculosis. Is it possible that a persistent trace of albumin in young adults is a frequent accompaniment of tuberculous disease even when there are as yet no strongly marked pulmonary symptoms?

We may, therefore, conclude from our study that the seriousness of albuminuria as a factor in mortality is established. The death rate is almost doubled during the few years succeeding the examination, but there is some variation according to the amount of albumin found and the age of the persons. The excess mortality results primarily from an increase in the death rates from Bright's disease, from tuberculosis and organic heart disease.

(*b*) Cases with albumin with casts.

This is a much larger group composed of 3,264 persons. They all showed casts, some hyaline, others granular, on microscopic examination. In view of the practice of the company, many of these persons were rejected outright after their first examination. We cannot, therefore, say that they all had persistent albuminuria, although in most cases several examinations were made and persistent albuminuria was found. These persons were exposed a total of 22,011 years of life which is an average of over six and one half years, a somewhat longer period than for the cases of albumin only. During this period, 452 deaths actually occurred. The death rate, therefore, was 20.5 per 1,000 for those having albumin and casts, as compared with 13.3 for those having albumin only. The following table gives the facts of mortality according to attained age for these cases:

TABLE III.

Mortality among rejected applicants showing albumin with casts, all quantities. Exposures, deaths and death rates per 1,000. Per cent. actual to expected mortality by various tables.

Attained age.	Exposure years.	Actual deaths.	Death rate per 1,000.	Per cent. actual to expected deaths.	
				Glover.	American men.
All ages.......	22,011.03	452	20.54	138	217
15 to 24.......	2,176.49	29	13.32	296	345
25 to 34.......	6,424.12	57	8.87	123	199
35 to 44.......	6,381.57	117	18.33	153	313
45 to 54.......	4,396.09	114	25.93	135	226
55 to 64.......	2,209.69	109	49.33	137	188
65 to 74.......	423.07	26	61.46	84	102

Obviously, the second group is much more severely impaired than the first and, this is true at each period of attained age except age 25 to 34.* When tested by the Glover Table for white males in registration cities, the ratio of actual to expected mortality was 138 per cent., as compared with 114 per cent. for cases showing albumin only and by the American Men Table, 217 per cent. of the expected, as compared with 183 per cent. The actual mortality is much higher than the expected on either table at virtually every age period. A striking excess is observed in the earliest ages, 15 to 24, where the mortality is 345 per cent. of that expected by the A. M. Table. The actual death rate is two and one half times as high as in the group with albumin only. This high death rate, however, applies only to those young people who show more than a faint trace of albumin with casts. There can be no doubt of the severity of the impairment of a trace or more of albumin with casts at these younger ages however doubtful may be the significance of a faint trace of albumin among very young people.

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* The writer is puzzled over the peculiar drop in the death rate at this period. Table IV shows that the drop is due mainly to the very low rate of mortality among those with a trace of albumin with casts.

As in the case with albumin only, there is a maximum point of excess mortality in the age period 35 to 44 and after that age, there is a diminution in that excess. Apparently, the seriousness of the impairment involved declines with advancing age. This conclusion must not be overstressed because of the relatively small number of persons under observation.

As before, it is necessary to analyze our data further to discover the rôle played by the amount of albumin and the age of the persons. This time it was possible to compare the three classes, namely, a faint trace of albumin with casts, a trace of albumin with casts, and a moderate or large quantity of albumin with casts, all three having sufficiently large exposures of lives to warrant analysis. The resulting figures point out that the excess mortality increases with the amount of albumin. The group showing only a faint trace of albumin with casts gives a mortality of 170 per cent. of that expected by the A. M. Table; those with a trace of albumin with casts, 256 per cent. of the table and, finally, those with a moderate or large quantity of albumin with casts, 482 per cent. of this same table. These facts are shown in Table IV.

The age relationships are not so convincingly clear. In the earliest age period, 15 to 24, only those with a faint trace of albumin with casts showed a favorable mortality. In fact, the ratio of actual to expected deaths was 91 per cent. On the other hand, those with larger amounts of albumin at this early age are among the most seriously impaired lives as indicated by the very high mortality rate. It is very striking indeed that an increase in the amount of albumin from a faint trace to either a trace or to a large quantity should increase the mortality five and six times, respectively. Yet, such are the facts and these bear out the opinions already very widespread among clinicians that slight traces of albumin in adolescents with or without casts are of no particular consequence but that traces or larger quan-

TABLE IV.

Mortality among rejected applicants showing albumin with casts according to amount of albumin. Exposures, deaths, and death rates per 1,000 by attained ages. Per cent. actual to expected deaths by various tables.

Attained age	Faint trace of albumin with casts.					Trace of albumin with casts.					Large or moderate quantity of albumin with casts.				
	Exposure years.	Actual deaths.	Death rates per 1,000.	Per cent. actual to expected deaths.		Exposure years.	Actual deaths.	Death rates per 1,000.	Per cent. actual to expected deaths.		Exposure years.	Actual deaths.	Death rates per 1,000.	Per cent. actual to expected deaths.	
				Glover.	Amer. Men.				Glover.	Amer. Men.				Glover.	Amer. Men.
All ages.......	11,261.29	196	17.40	106	170	8,789.18	187	21.28	157	256	1,960.56	69	35.19	282	482
15 to 24.......	831.41	3	3.60	71	91	1,075.17	20	18.60	370	465	269.91	6	22.23	461	545
25 to 34.......	2,914.76	21	7.20	99	162	2,792.09	18	6.45	89	144	771.27	18	25.10	346	563
35 to 44.......	3,305.73	51	15.43	128	263	2,485.42	47	18.91	158	324	590.42	19	32.18	275	575
45 to 54.......	2,452.41	53	21.61	113	191	1,683.67	43	25.54	134	226	260.01	18	69.23	367	620
55 to 64.......	1,484.49	56	37.72	103	151	622.76	46	73.86	205	301	102.44	7	68.33	194	280
65 to 74.......	272.49	12	44.04	65	82	130.07	13	99.95	143	176	20.51	1	48.76	67	83

tities of albumin with casts are very dangerous conditions. Our material suggests that the latter group includes many already suffering from Bright's disease and, in addition, others who have tuberculosis, the urinary findings being concomitants of the early stages of the lung disease. Out of a total of twenty deaths which occurred in the age period 15 to 24, seven were from tuberculosis or at a rate of 651 per 100,000.

After age 25, the death rates are almost uniformly high in the three groups. Among those with a faint trace of albumin with casts, the maximum excess of mortality as measured by the A.M. Table occurred at the age period 35 to 44. The death rate was two and two thirds times the expected. Among those with a trace, the ratio was three and one quarter times at the same age period. The number of persons with moderate or large quantities of albumin is small at each of the age periods but the mortality is obviously very excessive at all ages even if the actual figures are subject to much correction because of the smallness of the numbers.

The seriousness of the presence of albumin with casts is reflected also in the death rates for the various diseases. The mortality rate is high for virtually every important cause of death. Cancer shows a rate twice that found among Ordinary policy holders who are in every way comparable to this group except for the absence of urinary impairment at the time of their examination and acceptance by the company. Diabetes is almost three times as high. Tuberculosis is over three times as high as in the Ordinary experience. The extra mortality from this disease is not influenced so much by age as by the amount of albumin. We have already called attention to the suggestive relationship between incipient or masked tuberculosis and albuminuria in young adults. Most significant is the picture presented by the complex of the cardiovascular renal diseases. Apoplexy and cerebral hemorrhage give rates four times as high as in the company's ordinary experience; organic heart disease, over four times and Bright's disease, twelve times.

The incidence of heart disease increases with the amount of albumin and is much worse at the older ages than at the younger. Bright's disease, as might be expected, shows a rapidly increasing mortality with the increase in the amount of albumin, but this is much more pronounced among the older persons than among the younger ones. Over 40 per cent. of the deaths of persons showing a large amount of albumin with casts at the ages over 40 were from

Bright's disease and the death rate from this disease alone was 3,103 per 100,000. These persons would, therefore, appear to have been at the time of examination already suffering from Bright's disease or well on the road to this condition. But, even those with lesser amounts of albumin and those at the younger ages, are very seriously impaired lives as is shown by the death rates for the more important cause of death and, more particularly, for those which relate to the cardiovascular renal systems.

In the above discussion, no consideration has been given to the number and type of casts. The material at hand unfortunately did not give all the facts concerning the number of casts found on examination although we were able to distinguish cases according to the variety. There were about twice as many cases showing granular casts as hyaline. When the material was further subdivided according to the amount of albumin and according to age, the cases with hyaline casts were too few to permit any serious comparisons with the group containing granular casts. In general, it may be said that the cases showing hyaline casts on examination are not as highly impaired as the other, neither in the total mortality nor for Bright's disease, heart disease or tuberculosis.

This study has left much to be desired. It is realized that the group studied has not been a large one, but even more serious has been the relatively short period of time elapsed between the date of the examination and the closure of the investigation. The findings are presented with these limitations; yet, there is a very clear indication of the highly serious character of such impairments as albumin and albumin with casts. The gravity of the impairment varies directly with the amount of albumin and not quite so regularly with the advancing age of the applicant. The presence of casts adds materially to the gravity of the impairment and granular casts add more to the defects than do hyaline casts. At the older ages, the presence of casts, together with albumin, is indicative of a condition of the greatest seriousness. At the younger ages, the presence of a trace or more of albumin or of albumin with casts, suggests the existence of tuberculosis in many cases.

A TOXIC SUBSTANCE OBTAINED BY GROWING HEMOLYTIC STREPTOCOCCI IN A SPECIAL MEDIUM.*

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Invasion by the hemolytic streptococcus is responsible for a number of different diseased conditions. Thus, we have "septic sore throat," tonsillitis, bronchitis and broncho-pneumonia—to name but a few of the more common diseases in which the hemolytic streptococcus plays a prominent part. In addition, hemolytic streptococci are responsible, as secondary invaders, for grave complications in other diseases, of which measles may be taken as an example. The severity of the systemic reaction is a striking characteristic of all these infections and, where a fatal termination results, it is often difficult of explanation by the extent of the pathologic process or the apparent degree of the infection.

In addition to these clinical considerations, there are other points which suggest that at least a part of the pathogenic power of hemolytic streptococci is to be ascribed to specific intoxication. Thus, when mice are injected intraperitoneally with several times the lethal dose of a virulent strain, death usually takes place within a few hours. Whether death occurs in two hours or is delayed for twenty-four hours or longer, autopsy reveals little to explain the mechanism of the pathogenic action. The peritoneum contains little or no exudate. Organisms can be recovered from the heart's blood, but smears and cultures frequently reveal the absence of an overwhelming septicemia. Autopsy findings in man are correspondingly unsatisfactory in revealing the manner in which this organism produces its fatal effects.

The present study was undertaken, therefore, in the hope of determining more definitely the mechanism of the pathogenic action of the hemolytic streptococcus.

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Experimental.

The Organisms.—The source and laboratory numbers of the nine strains of hemolytic streptococci included in this study were as follows: Strains 4 and 64 were isolated from the sputa of cases of broncho-pneumonia, the first in January, 1920 and the second in November, 1918; strains 6 and 16 were recovered from throat cultures of cases of acute tonsillitis in February, 1920; strains 9, 34, 41 and 43 came from throat cultures of influenza patients, January, 1920; strain 170 was recovered from a throat culture of a case of acute bronchitis, October, 1918. All of these strains produced typical beta hemolysis on blood agar plates and their morphology, fermentation reactions and insolubility in bile placed them in the hemolytic streptococcus group. These particular strains were selected on account of their original virulence for mice and because it was found possible to raise their virulence by means of animal passage. The final virulence of all the strains was such that from 0.001 cc. to 0.0001 cc. of an 18-hour broth culture killed white mice within from twenty-four to forty-eight hours.

Experiment I illustrates the fact, already referred to, that streptococcus infections may be fatal with comparatively scant growth of the organisms within the host.

Experiment I.—Mouse 1: This mouse was given, intraperitoneally, 0.001 cc. of an 18-hour blood broth culture of strain 9. Death occurred in thirty hours. There was a slight exudate in the peritoneum. Films from the heart's blood showed only an occasional streptococcus.

Mouse 2: Five tenths cc. of a blood broth culture of strain 9 was injected into the peritoneal cavity. The mouse was dead four hours later. The peritoneum was dry. The mesenteric and visceral peritoneum was moderately congested. Films from the heart's blood showed very few organisms. Cultures made from the peritoneal cavity and heart's blood were positive.

The results obtained in Experiment I corroborate the statement made above, that infections by the hemolytic streptococcus may be fatal in the absence of an extensive multiplication of the organism in the tissues of the host. This was true in both instances here noted, one mouse receiving a 30-hour lethal dose and the other mouse 500 times this amount. It is indicated, therefore, that the organism produces a very active poison on multiplication within the tissues of the host, since only a slight growth was necessary to cause death, and since sterile filtrates from the infecting cultures were devoid of toxic action, 2 cc. not being fatal.

Evidence of the production of toxic substances in infected animals leads one to believe that the hemolytic streptococcus (as well as many other pathogenic bacteria) would do the same in vitro if it were grown under the proper conditions. . An attempt to devise a medium in which this organism would produce filtrable toxic substances resulted in the preparation of the following medium which was used in the experiments described below: 1 per cent. peptone, 1 per cent. di-sodium phosphate and 0.5 or 1 per cent. glucose are added to an ordinary meat infusion made with distilled water, and the reaction adjusted to a PH of 8.0 to 8.2. The medium is tubed, about 10 cc. to a tube, and autoclaved at ten pounds' pressure for ten minutes. A fragment of sterile rabbit kidney and 1 cc. of defibrinated sheep or rabbit blood are now put in each tube. The medium is then ready for use and, to obtain the best results, should be inoculated at once, without incubation to determine sterility. Contaminated tubes can be detected and discarded as they appear.

In this medium the hemolytic streptococcus grows readily and abundantly. Cultures grown in it for a period of 18 to 36 hours were highly virulent for mice and killed fully as rapidly as when grown in plain meat infusion broth. Occasionally filtrates of such cultures incubated for this length of time will kill when injected in 1 or 2 cc. amounts, but not with any degree of certainty. After 48 hours' incubation there occurs a very marked increase in the toxicity of the centrifugalized or filtered culture and this increases up to 72 or 96 hours, when it begins to decrease. This time curve of the rise and fall of the toxicity of the cultures in this broth has been characteristic of the strains studied. Usually the maximum toxicity is obtained in about 72 hours, when from 0.1 to 0.2 cc. of filtrates from cultures kill white mice within 24 hours.

PROPERTIES OF THE TOXIC SUBSTANCE.

Effect of Filtration.—The passage of cultures through Mandler filters does not destroy the toxic action of the filtrate. While the supernatant fluids of cultures centrifugalized at high speed still contain a few organisms the number present has not been found to interfere seriously with the study of the toxic substance. This substance apparently lacks the characteristic aggressive action of most true toxins known at present and consequently the presence of a few streptococci in the fluid injected does not lead to a fulminating infection. This point will be referred to later in detail. The Mandler filtrates were about one half as toxic as the supernatant fluid. This indicates that a portion of the toxic substance is held back or destroyed by filtration.

Relation of Acidity to Toxicity.—It is well known that streptococcus cultures produce large amounts of acid, and, while the initial reaction of the medium and the buffer action of the phosphate tend to control this acidity, most cultures in three days' time reach a PH of 6.5 to 6.0, and occasionally a higher degree of acidity develops. It was possible, therefore, that the toxic action might be due to the acidity, but neutralization of the fluid with sodium hydroxide does not alter the toxicity.

Specificity.—It was also possible that other organisms grown in this medium, or the medium alone, might display the same toxic effect. However, filtrates of the sterile medium incubated three days, and filtrates of cultures of non-hemolytic streptococci, pneumococci, staphylococci and typhoid bacilli grown in the medium showed no toxic action when injected, even in large amounts, into mice. This is in marked contrast to cultures of hemolytic streptococcus strains, where 0.1 cc. of the filtrate is often fatal to mice in less than twenty-four hours.

Pathogenicity.—As mentioned above, this product is toxic for mice when injected intraperitoneally. There appears to be a definite relation between the amount injected and the time of death. Following the injection of amounts as large as 1 to 2 cc. death sometimes occurs in as short a time as one hour. This is the shortest interval that has been observed with intraperitoneal injections. Following acutely fatal doses, death is apparently due to respiratory failure, the heart having been observed to beat for several minutes after respiration has ceased. When smaller amounts are injected and death is delayed the symptoms are more prolonged. Prostration occurs and the respirations become more and more difficult and labored until the time of death. Autopsy shows a remarkable absence of any gross pathological condition.

The intravenous injection of mice produces almost instantaneous death, the animal often being dead before it can be released following the injection. Even such small amounts as 0.01 cc. have sometimes caused death. If the injection is not immediately fatal the incubation period is variable. The intraperitoneal method gives more satisfactory results, the incubation period and the final results being more uniform.

A small number of rabbits and guinea pigs were injected, but the effect of the toxic substance on these animals is irregular, some being apparently very susceptible and others relatively resistant. The intravenous injection into rabbits of 1 cc. per kilogram of body weight has caused death. The incubation period is usually short, as is the case with mice injected intravenously. The incubation period has been known to vary, however, from five minutes to eighteen hours, and many rabbits do not succumb to the injection. Death is preceded by convulsions and extreme respiratory distress.

Thermolability.—The toxic action of the filtrates is impaired by heating to 55° C. for 30 minutes and is completely destroyed by heating to 62° C. for 30 minutes.

Stability.—The filtrates rapidly lose their toxic properties even when kept sealed and in a cold dark place. Some specimens deteriorate as much as 50 per cent. in a few days' time in the ice box.

Relation of Virulence to Toxicity.—Roughly, the production of the filtrable toxic substance corresponds to the virulence of the strain of hemolytic streptococcus used. The increase of virulence of a strain by animal passage usually is accompanied by an increase in the production of the toxic product, although one strain (64) which was relatively avirulent for mice, produced a potent poison in vitro.

IMMUNIZATION EXPERIMENTS.

Immunization of Rabbits.—The injection into rabbits of sterile filtrates of cultures containing this toxic substance causes the production of neutralizing and protective substances in their serum. The rabbits respond whether injected intravenously or subcutaneously. The following protocols are illustrative of the immunization process.

PROTOCOL 1.

Immunization of rabbits with the filtrates.

Date.	Rabbit 1, strain 9, intravenous.	Rabbit 2, strain 41, intravenous.	Rabbit 3, strain 9, subcutaneous.	Rabbit 4, strain 41, subcutaneous.
4–27–20........	0.25 cc.	0.25 cc.	0.5 cc.	0.5 cc.
4–28–20........	0.25 cc.	0.25 cc.		
4–29–20........	0.5 cc.	0.5 cc.		
4–30–20........			0.5 cc.	0.5 cc.
5– 2–20........	0.5 cc.	0.5 cc.		
5– 3–20........	0.5 cc.	0.5 cc.	1.0 cc.	1.0 cc.
5– 4–20........	1.0 cc.	1.0 cc.		
5– 7–20........	0.5 cc.	0.5 cc.	1.0 cc.	1.0 cc.
5– 8–20........	1.0 cc.	1.0 cc.		
5– 9–20........	1.0 cc.	1.0 cc.		
5–10–20........			2.0 cc.	2.0 cc.
5–17–20........	Bled	Bled	Bled	Bled

The sera obtained from the rabbits of Protocol 1 were tested with respect to their neutralization and protection properties, both against the toxic filtrates and infection with the cultures. Protocols 2, 3, 4 and 5 illustrates the type of experiment used.

PROTOCOL 2.

Neutralization of the poison (Strain 41) in vitro.

Mouse.	Filtrate, strain 41.	Immune serum, rabbit 2.	Normal rabbit serum.	Results.
1.......	0.1 cc.	0	0	Dead in 26 hrs.
2.......	0.1 cc.	0	1.0 cc.	Dead in 28 hrs.
3.......	0.5 cc.	0.5 cc.	0	Survived.
4.......	0.5 cc.	0.25 cc.	0	Survived.

In Protocol 2 the serum and filtrate were mixed and kept at 37° C. for one hour before injection. The mixtures were injected into the peritoneal cavity. It is seen that 0.25 cc. of serum neutralized five lethal doses and that the same amount, or even twice the quantity of normal rabbit serum, had no neutralizing effect upon the toxic filtrate.

Protocol 3 illustrates the protective action of the serum of the immunized rabbits when the serum and the filtrates are separately injected. The serum was injected 8 hours prior to the filtrate.

PROTOCOL 3.

Neutralization of the filtrate in vivo.

Mouse.	Filtrate, strain 9.	Immune serum, rabbit 3.	Normal rabbit, serum.	Results.
1.......	0.2 cc.	0	0	Dead in 20 hrs.
2.......	2.0 cc.	0	1.0 cc.	Dead in 4 hrs.
3.......	2.0 cc.	1.0 cc.	0	Survived.

Protocol 3 shows that 1 cc. of the immune serum protected against ten lethal doses of the filtrate and that the same amount of normal rabbit serum was without effect. Other similar experiments showed that from 0.2 cc. to 0.05 cc. of the sera of the immunized rabbits protected against a lethal dose of the homologous filtrate.

Proportional Neutralization.—In view of the fact that 3 cc. is about the maximum quantity that can be injected into a mouse at one time and since this amount of filtrate would contain hardly more than twenty lethal doses, the following experiment was devised in order to test the proportional neutralizing action of the immune serum on multiple lethal doses of the toxic substance. Thirty cc. of filtrate 9, representing 200 fatal doses, was mixed with 8 cc. of serum 9, 200 neutralizing doses. This mixture was allowed to stand at room temperature for one hour and then 3 cc. were injected intraperitoneally, into each of six mice. The results are shown in Protocol 4.

PROTOCOL 4.

Neutralization of proportional multiple doses of the filtrate.

Mouse.	Amount of Mixture.	Results.
1	3.0 cc.	Survived.
2	3.0 cc.	Survived.
3	3.0 cc.	Dead in 36 hrs.
4	3.0 cc.	Survived.
5	3.0 cc.	Dead in 48 hrs.
6	3.0 cc.	Survived.

It is seen that four of the six mice survived and that the other two lived more than 24 hours in spite of the fact that the mixture which each mouse received contained more than fifteen lethal doses of filtrate. This seems to show that multiples of the toxic filtrate up to at least 200 are quite effectively neutralized by equal unit proportions of the immune serum.

Protection Against Infection.—The following protocol was devised to determine the effect of the serum on infection with cultures of the different strains of hemolytic streptococci included in the study. In this experiment all injections were intraperitoneal and the serum was given 18 hours prior to the culture.

PROTOCOL 5.

Protection against infection.

Mouse.	Culture, strain 9.	Immune serum, strain 9.	Results.
1	0.0005 cc.	0	Dead in 20 hrs.
2	0.0005 cc.	0	Dead in 18 hrs.
3	0.05 cc.	0.5 cc.	Survived.
4	0.05 cc.	0.5 cc.	Suvrived.

This protocol shows that the serum of rabbits immunized with the toxic filtrate protected mice against at least fifty fatal doses of the whole culture. This seems to point to a definite specific relationship between the toxic substance and the organisms.

The Relation of Protection and Serological Grouping.—By means of agglutination and protection experiments with an antibacterial serum produced with strain 9 it was found that at least two serological groups existed among the strains included in this study. Thus, the strain 9 serum agglutinated and protected against strains 4, 6 and 9, while it neither agglutinated nor protected against strains 41, 64 and 170. It was, therefore, of interest to see if this grouping extended to the toxic substances and their immune sera. For this purpose rabbits were immunized with the filtrates from strains 6, 9 and 41 respectively and their sera used in protection tests with filtrates from cultures 4, 6, 9, 41 and 64. The sera and filtrates were mixed in vitro and kept at room temperature one hour before they were injected. The details of the experiment are given in Protocol 6.

PROTOCOL 6.

Cross protection tests with the toxic filtrates and their respective immune sera.

Mouse.	Filtrates.	Immune serum.	Results.
1......	0.5 cc. strain 9	0.5 cc. strain 9	Survived.
2......	0.5 cc. strain 6	0.5 cc. strain 9	Survived.
3......	0.5 cc. strain 4	0.5 cc. strain 9	Survived.
4......	0.5 cc. strain 6	0.5 cc. strain 6	Survived.
5......	0.5 cc. strain 4	0.5 cc. strain 6	Survived.
6......	0.5 cc. strain 9	0.5 cc. strain 6	Survived.
7......	0.5 cc. strain 41	0.5 cc. strain 41	Survived.
8......	0.5 cc. strain 64	0.5 cc. strain 41	Survived.
9......	0.5 cc. strain 9	0.5 cc. strain 41	Dead in 16 hrs.
10......	0.5 cc. strain 41	0.5 cc. strain 9	Dead in 8 hrs.
11......	0.5 cc. strain 4	0.5 cc. strain 41	Dead in 16 hrs.
12......	0.5 cc. strain 41	0.5 cc. strain 6	Dead in 8 hrs.

The results of Protocol 6 show that the sera of the rabbits immunized with filtrates from the strains (two strains) of one serological group protected against filtrates from all the strains of this group but not against filtrates from two strains of the other group. In like manner the serum from a rabbit immunized with the filtrate from a strain of the other group protected against its own filtrate and the filtrate from another strain of this group and failed to protect against filtrates from the first group. It appears then that filtrates from different serological groups have correspondingly different antigenic properties. This part of the work, however, needs to be extended.

Discussion.

The development of the poisonous substance described above has been found to depend upon the cultivation of the hemolytic streptococcus in a special medium. The use of ordinary media of various kinds leads to negative results. The use of special media for the demonstration in vitro of toxic products of the growth of pathogenic bacteria has been found to be of value in a number of instances. Clark and Felton (1) (1918) have suggested that a filtrable toxic substance is produced by the growth of hemolytic streptococci in blood diluted with Locke's solution. Bull and Pritchett (2) (1917) found that fresh tissue was an important constituent of the medium in demonstrating the production in vitro of the toxin of *B. welchii,* and Robinson and Meader (3) (1920) have found that such a procedure enhances the toxicity of the broth in which *B. diphtheriae* is grown.

At present only conjecture can be made concerning the purpose served by the different constituents of our medium. Certain precautions are, however, necessary. Distilled water should be used in the preparation of the meat infusion, since it has been found that certain specimens of tap water have an unfavorable action on the production of the poison. No sodium chloride is added since this salt seems also to have an unfavorable influence upon the production of the toxic substance. The addition of di-sodium phosphate is apparently necessary, but its influence is not entirely clear. It probably acts as a buffer in limiting the amount of free acid present in the culture fluid, since the toxicity of the medium decreases as the acidity rises. The presence of blood is not absolutely essential to the maximum production of the poisonous product, but a medium containing 10 per cent. of defibrinated sheep or rabbit blood has given more constant results than the plain medium. The use of fresh tissue is important. The tissue must be fresh and the medium used immediately.

In describing this filtrable toxic substance care has been taken to avoid the use of the term "toxin." The word "toxin" has come to be regarded as defining specific substances, generally of bacterial origin, which conform to definite criteria, such as high toxicity, a definite and characteristic relation between the so-called Lo and the L + doses, definite antigenic properties and certain other characteristics of equal definiteness. While the toxic product which has been described for the hemolytic streptococcus possesses certain of the properties of a true toxin, such as the neutralization of large amounts by proportionate amounts of immune serum, and the production of immune sera which protect against infection with the cultures, it has been impossible to demonstrate other characteristics of true toxins. The toxic substance of the hemolytic streptococcus lacks high potency, although it compares favorably in this respect with the toxin of

B. welchii. It causes no local lesion and consequently it has been impossible to determine an Lo dose with any consequent differences between the Lo and the L + doses. Furthermore, it apparently lacks the typical agressin action which is a striking characteristic of all other known toxins. The injection of the toxic substance together with, or preceding, the injection of the streptococci themselves, has no marked influence on the course of the infection, aside from that which can be ascribed to the toxic action of the fluid itself.

The specificity of the toxic filtrates seems to have been established. As antigens they stimulate the formation of antibodies which neutralize their toxic action and also the homologous cultures. Further evidence of specificity is afforded by the fact that immune sera produced with filtrates from one serological group do not neutralize the filtrates from another group.

It is impossible to state definitely at the present time what part, if any, this poisonous product plays in natural infections with hemolytic streptococci, but both clinical and experimental evidence seems to point to the action of some such product as at least a part of the process of natural as well as experimental infections with this organism.

SUMMARY.

A special medium has been described in which a specific toxic substance has been produced during the growth of certain strains of hemolytic streptococci.

This toxic product is filtrable and the filtrates have a definite pathogenic action when injected into mice, rabbits and guinea pigs.

The poison possesses definite antigenic properties and the sera of rabbits immunized with such toxic filtrates gives protection both against infection with the cultures and against intoxication with the filtrates.

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STUDIES ON THE LIFE HISTORY OF VAHLKAMPFIA PATUXENT N. SP., PARASITIC IN THE OYSTER, WITH EXPERIMENTS REGARDING ITS PATHOGENICITY.*

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Plates IX to XI.

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Introduction.

The interest in protozoa has been steadily increasing in the last few years. Many diseases have been found to be caused by various protozoa and still other protozoa are regarded as highly suspicious. Indeed the probable cause of many illnesses not already accounted for is thought by competent research workers to be due to protozoa whose pathogenicity has not yet been determined.

There are many cases reported of persons becoming ill from eating raw oysters. So far the cause of these illnesses has not been determined. It seemed timely therefore, from a public health point of view, to make a protozoan survey of the oyster beds of Maryland with the object of determining what protozoa are found in the oysters and whether they are pathogenic.

Our present knowledge of the protozoan parasites of the oyster is very scant. There is, however, a rather large amount of literature on the spirochaetes inhabiting the stomach and intestine and especially the crystalline lens of the oyster. Certes (1882) was the first person to describe this spirochaete which he called *Trypanosoma balbianii,* to which "Certes" has since been added. He found it in *Astrea edulis* and *Astrea angulata* on the west coast of France. In 1883 Möbius published observations on this same *Trypanosoma balbianii* Certes and said that he had observed it first in 1869 in the oysters of Schleswig Holstein. In the same year (1883) Ryder, working at Woods Hole, notes the presence of these spirochaetes in *Astrea virginica,* and observes that "upon eating these same individuals, known to be infected with parasites, no inconvenience was experienced, showing that these organisms, whatever they may be, are probably harmless to man.'' Since then much has been published on this particular parasite of the oyster.

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In 1914 I published an account of the life history of an amoeba parasitic in the digestive tract of the oyster. Unfortunately at the time I could not test its pathogenicity and the strain of amoebae was lost while I was away doing war work.

In the fall of 1919 I began a protozoan survey of the oyster beds of Maryland. Soon after starting this work, I found a rather large amoeba in the digestive tract of the oysters from the Patuxent river. This amoeba was isolated and cultured and forms the basis of the present work.

The purposes of this work are to study the life history of this amoeba, to observe its reactions to different environment, and to test its pathogenicity. So far the study of the life histories of the forms parasitic in invertebrates has proved most helpful in the interpretation of the phenomena observed in the life histories of the amoebae parasitic in man.

Method.

In accordance with the rules adopted by the Committee on Standard Methods of Shell Fish Examination of the American Public Health Association, the oysters were first scrubbed and thoroughly washed with running water in order to free them from dirt and from small mussels growing on them. They were then dried in the air and the edges of the shell, where the oyster was to be opened, were flamed in a gas burner. Then the edges were cracked off with a hammer which had been flamed, and a clean scalpel, which had also been flamed, was inserted and the adductor muscle cut, so that it was easy to remove one of the shells.

The liquor of the oyster was then drawn off with sterile pipettes and the oesophagus and later the anus were cut open with sterile scissors. The contents of the stomach and intestine were removed with a fine sterile pipette and streaked out on agar plates. These were kept in moist chambers at room temperature.

Culture media.

Solid media.—As a basis for the solid media 1.5 per cent. agar agar was used. This per cent. of agar had been shown (Hogue, 1917) to be the best for the growth of amoebae. It was usually made up with .7, 1, or 1.5 per cent. sodium chloride solution. The .7 per cent. solution was mostly used as it was kept in stock in the laboratory and the amoebae grew as well on it as on any of the other sodium chloride solutions. In fact all three solutions gave equally good results. More than 1.5 per cent. of sodium chloride was not used with these amoebae, although with *Vahlkampfia calkensi,* also from the oyster (Hogue, 1914), as high as 3 per cent. was used. To all these sodium chloride media .4 per cent. peptone was added. When the bacterial growth became too luxurious, and was killing out the amoebae, the amount of peptone was reduced to .2 per cent. This kept down the rapid growth of the bacteria and gave the amoebae a chance to regain their normal size.

Neutral red was added to some of the sodium chloride agar agar tubes. It was hoped that the bacteria on which the amoebae live would take the stain. This however did not happen.

Agar agar 1.5 per cent. was also made up with Locke solution after the following formula:

Sodium chloride	0.9	gram
Calcium chloride	0.024	gram
Potassium chloride	0.042	gram
Sodium bicarbonate	0.02	gram
Dextrose	0.25	gram

Several attempts were made to grow amoebae on agar agar made up with distilled water to which .4 per cent. peptone was added. The amoebae did not live on this medium.

Liquid media.—Two liquid media were used: Locke solution and ovomucoid. To make the ovomucoid the white of one hen's egg was added to 200 cc. of 1 or 1.5 per cent. sodium chloride solution. This was well shaken up and cooked on a hot water bath for half an hour. It was then filtered through cotton into test tubes. To each test tube was added a loop full of the yolk of the hen's egg (Wherry, 1913). It was then autoclaved.

All the media were sterilized in the autoclave for twenty minutes under fifteen pounds of pressure. The solid media were poured into sterile Petri dishes. Slants were occasionally used, though the amoebae multiply so rapidly that a test tube did not offer a sufficiently large surface for growth and hence required too frequent transplanting to be practicable.

Stab cultures.—Experiments were made with stab cultures. Test tubes with 1.5 per cent. agar, .4 per cent. peptone, and 1, and also 1.5

per cent. sodium chloride solution were inoculated by the stab method with three pure lines. In all cases the amoebae grew luxuriously on the surface of the agar only. The growth of bacteria in the agar was slight and filiform in character.

TECHNIQUE.

A drop of .7 per cent. sodium chloride solution was put on a clean slide and a loop full of amoebae was added. The slide was allowed to remain in a moist chamber from ten minutes to many hours. This gave the amoebae time to attach themselves to the slide and to assume a normal shape before being fixed.

To fix the amoebae the slides were dropped face downward into hot Schaudinn solution and allowed to remain there for about three minutes. They were then removed to the alcohols and stained twenty-four hours in Heidenhain's iron-haematoxylin, .5 per cent. Occasionally safranin was used and with it Licht-grün or acid-violet for cytoplasmic stains. Mann's stain and Rosenbusch's stain both gave very interesting nuclear pictures and brought out many structures which were not clear with Heidenhain's iron-haematoxylin. Dobell's iron haematin was also useful.

Thomson and Thomson (1916) agar slides were used with very helpful results. Melted agar, made up with Locke or .7 per cent. sodium chloride solution, was poured onto a clean slide and then most of it was drained off, leaving only a very thin film of the medium on the slide. This was inoculated with amoebae. The slides were kept in a moist chamber for varying lengths of time and then fixed in cold Schaudinn, stained with iron-haematoxylin, dehydrated and mounted in balsam. It was an uncertain procedure, as the agar with its growth, occasionally floated from the slide and was henceforth useless. Great care had to be taken not to leave the agar too thick, or the oil immersion could not be used, and also not too thin, or it would dry up. The amoebae did not adhere as well to the agar agar as to the glass slide and many were lost in making the preparation.

It was found advantageous to add a loop full of sterile .7 sodium chloride solution to each transplant so as to make the medium a little more moist and so aid the amoebae in spreading out over the plate. When all went well, the results were most satisfactory as you had an exact picture of the amoebae as it was living its life cycle, quite undisturbed by being transferred from the solid medium to the slide or cover slip.

"PURE LINES."

As soon as the amoebae appeared in any considerable numbers on the agar plates, they were transferred to a new Petri dish and allowed to spread out until they were quite far apart. Then, as I described in 1917, a small block of agar agar, containing only one amoeba, was cut out and transferred to a larger piece of sterile agar which was kept in a moist chamber. If these lived and multiplied they were transferred to an agar agar Petri dish. After that subcultures were made with a platinum loop where many amoebae were taken at a time, but as they now were a "pure line," i.e., all derived from one individual, it did not matter how many were transplanted.

BACTERIA.

Bacteria were taken with each transplant. Unfortunately a "pure mixed culture" was not obtained as the amoebae rarely encysted so that cysts could not be obtained in sufficient number to wash them with acids in order to free them from the bacteria. A Barber pipette was not at my disposal when the work was begun so that I could not isolate a single bacterium. However, through the kindness of the bacteriological department I had the bacteria which were growing with one pure line carefully studied and identified. Three different bacteria were present, the prevailing one being a bacillus. None of the bacteria were pathogenic.

GROWTH.

When these sodium chloride media were inoculated with amoebae in the center of the Petri dish, the amoebae spread out quite evenly in ever widening circles over the whole dish, always following the growth of the bacteria. Where the bacterial growth stopped there would be a ring of amoebae five or six deep. Beyond this ring the amoebae were few and widely scattered. The whole Petri dish is usually covered with amoebae in a week's time. Gradually most of these amoebae go to pieces, though a few smaller ones remain scattered about among the debris, often grouped in masses. This is quite different from *Amoeba calkensi* (Hogue, 1914) where many of the amoebae encysted in a week's time.

When the Petri dish is swarming with bacteria, the amoebae do not thrive. They seem to be smothered by the bacteria and the bacterial waste products. Cropper and Drew (1914) say that concentration of the bacterial waste causes amoebae to encyst, but in my cultures, when the bacteria predominate, the amoebae become very small in a day or two, and then usually disappear entirely. Occasionally they emerge along the sides of the bacterial growth and thus work their way out beyond the dense mass of bacteria.

When this balance between amoebae and their food supply is disturbed the best way to bring them again into equilibrium is to transplant them onto Locke agar plates where the bacteria do not multiply so rapidly. Here the amoebae almost at once regain their normal shape and size.

THE TROPHOZOITES.

1. Movement.

These amoebae have a very characteristic motion. There is a large clear anterior ectoplasmic pseudopodium (Figs. 1 and 9) distinctly marked off from the granular endoplasm. This pseudopodium flows gradually forward, keeping much the same size and shape. Behind it comes the endoplasm. The motion of the ectoplasm is much like that of the sea, as it spreads up on the sand after the waves have broken. At times in certain media, the amoebae send out explosive pseudopodia which go first around one side of the animal and then around the other, like blebs. This, however, is unusual and mostly indicates the setting in of death processes.

2. Morphology.

The amoeba has several characteristic forms. One of the most common of these is a broad fan shaped animal (Fig. 1) having a wide anterior end with a broad flat ectoplasmic pseudopodium, and a pointed posterior end from which usually extend small fine pseudopodia. This form is frequently wider than it is long. When the amoeba is in rapid motion, it elongates and the anterior pseudopodium breaks up into several smaller ones (Fig. 9).

The size of the amoeba varies much according to the age of the culture. For the first three or four days after a Petri dish has been inoculated with amoebae, they are a fairly uniform size, averaging 21 μ in length and 13 μ in width. After that the size varies greatly and you may find very small amoebae, not measuring more than 1.6 μ in length (evidently the result of budding), and giant multinuclear forms (Fig. 6), some of these measuring as much as 143.3 μ in length.

The endoplasm is full of small granules and large refractile granules. The large ones never pass out into the ectoplasm. Many of them take the neutral red stain and are probably neutral red granules. Occasionally in the old cultures the small granules pass out into the ectoplasm. This is usually a herald of the setting in of abnormalities leading to death. The ectoplasm disappears and probably mixes in with the endoplasm when the amoeba encysts, and also when it rounds up in response to mechanical stimulation.

Vacuoles.

There is no contractile vacuole, as is to be expected in a salt water protozoon. However there are other vacuoles present. Some of these are food vacuoles and are filled with bacteria and finely granular material. The number of these vacuoles varies greatly. Some amoebae will have none, others will have their endoplasm full of them (Figs. 6 and 11). The size of the vacuoles varies from small spherical ones to a large one practically filling the entire animal and pushing the nucleus off to one side.

The large vacuoles change shape with the changing form of the amoeba. Occasionally when the amoeba is elongating or constricting and pressing against the large vacuole, it will constrict and become divided into two smaller vacuoles. Its contents also divide (Fig. 6).

Again two of the vacuoles fuse to make one. Both these processes have been observed in the living amoebae. Still another large vacuole was seen to open and to expel its granular contents to the outside. Unless this had been observed under high power it would have appeared like a contractile vacuole, but the solid content being expelled made this interpretation impossible.

These amoebae have probably taken in more water with their food than they can assimilate and the equilibrium of their cytoplasm has been disturbed, resulting in these numerous vacuoles. The chromatin complex in such amoebae is sometimes disturbed. Figure 11 shows the nucleus broken up into several small masses. This nuclear disturbance does not seem to be fundamental for this amoeba is in the process of dividing.

In the living amoeba these vacuoles with their granular content are often hard to distinguish from endogenous buds. In the stained preparations the difference is clear. The buds (Figs. 2, 5, 9, 10, 12) have a definite outline and a nucleus while the granular contents (Figs. 6, 11) of the vacuoles are irregular in outline and without a nucleus. Some of the vacuoles, especially the smaller ones, are often filled only with clear liquid without granular content. They do not take the glycogen stain with Kofoid's (1919) iodin-eosin formula.

Nucleus.

The nucleus is usually spherical (Figs. 2, 3) and contains a large slightly eccentric karyosome which is also spherical, unless preparing for division or reconstructing after division (Fig. 13*a*). With iron-haematoxylin the karyosome stains a deep blue, though it may contain one or more clear linin spaces (Figs. 6. 9, 13*a*), as has been noted for other amoebae by various investigators. With this stain there is a definite nuclear membrane and often, when the specimens have not been too much destained, the peripheral chromatin appears on the nuclear membrane as small granules (Fig. 2) and occasionally as larger granules (Figs. 5, 31) resembling those of *Entamoeba coli.* Between the nuclear membrane and the karyosome is a clear area.

When safranin and Licht-grün are used the karyosome is brilliant red and the surrounding area a vivid green.

Mann's stain and especially Rosenbusch's stain brought out other details. With Rosenbusch's stain the karyosome appears as a dark blue ring (Fig. 3) with a light grey center. The peripheral chromatin appears on the membrane, while surrounding the karyosome is a light grey area. It appears as a structureless ground substance and of irregular outline, often with fine lines passing out into the clear area which reaches to the nuclear membrane. It appears as some material which is diffusing out from the karyosome. In a recent paper (1920) Huxley has described a '' granular band '' in the nucleus of *Amoeba clavellinae.* He says at times the band appears structureless and is of varying width. This area seems to closely resemble the light grey area which I have just described for this amoeba. This light grey substance is in the clear area which in the haematoxylin slides fills up the space between the karyosome and the nuclear membrane.

Binary fission.

Division is by promitosis, the karyosome dividing into two parts which form the polar masses. This takes place within the nuclear membrane.

Prophase and metaphase.

The first indication of division is the elongation of the karyosome and with it the stretching out of the nuclear membrane (Fig. 18).

The peripheral chromatin granules soon begin to move around on the nuclear membrane to a point opposite the middle of the karyosome (Fig. 19). Here they become massed together and press against the karyosome so as to indent it. The karyosome thus becomes bean shaped (Fig. 19) and later dumb-bell shaped (Fig. 20), the future polar masses being the ends of the dumb-bell. While this is going on the spindle is forming. Figure 21 shows the fibres of the spindle connecting the chromatin polar masses, in side view. A late stage of this division of the karyosome is seen in front view in Figure 22, where a narrow connecting link is still seen between the polar masses of chromatin.

There is an equatorial plate of chromatin (Figs. 23, 24) which in all probability comes in from the peripheral chromatin. Definite chromosomes were not distinguishable, nor have I been able so far to find later stages in the division of the equatorial plate.

Anaphase.

The anaphase is recognizable by the constriction of the spindle and of the nuclear wall and by the broadening out of the polar masses at right angles to the spindle (Figs. 25, 26).

Telophase.

The constriction of the spindle continues until it is divided into two parts (Fig. 27). Then the nuclear wall divides (Fig. 29) and reconstruction begins at once.

Reconstruction.

The process of reconstruction goes on very rapidly so that it is often difficult to find the stages, even though you know there is much division taking place. But this is true of all the stages in division. The polar masses of chromatin quickly fuse with the chromatin from the equatorial plate, which is always less in quantity (Figs. 13*a*, 28, 30). The spindle material between these two different masses of chromatin passes out to the periphery (Fig. 30). Some chromatin material also goes to the periphery to become the peripheral chromatin. In a few specimens (Figs. 31, 32, 33) one sees fine lines and slender strings of chromatin granules passing from the karyosome out to the periphery. As soon as the chromatin from the old karyosome and from the equatorial plate have fused there is formed an irregular shaped karyosome—often oblong—sometimes pointed as is shown in Fig. 29. Figures for this stage resemble some of those given by Dobell (1919) for *Endolimax nana.* His Figs. 18, 22, 24 and 25 look very much like stages in reconstruction after promitosis. Again the two masses of chromatin may be slow in fusing. In this case the peripheral chromatin is seen far from the chromatin mass, but attached to it by the slender spindle threads (Fig 13*a*). This is found on slides where there are many multinucleate forms and where nuclear division is going on very rapidly. These two masses of chromatin of different sizes might easily be interpreted by the inexperienced investigator as the giving off of chromatin from the nucleus, the small amount coming from the equatorial plate being interpreted as the part which would form chromidia. But this interpretation cannot hold as the later stages show the smaller amount of chromatin being drawn into the larger amount and fusing with it (Fig. 28). Occasionally I found a slide where the majority of the amoebae were in this stage of reconstruction. It always occurs in the young cultures. Sometimes one nucleus will reconstruct much more rapidly than the other, so that there will be two stages of reconstruction in one individual, but this is rare (Fig. 29). In all the stages of division it was very difficult to see the polar cap. Figure 28 shows it in a reconstructing nucleus above the polar mass of chromatin.

Cytoplasmic division.

Division of the nucleus is normally complete before the amoeba begins to divide into two new individuals (though at this time reconstruction may not be complete). During division the amoeba moves about freely. This is contrary to Jepp and Dobell's (1918) observations that "most amoebae when undergoing division are rounded and at rest." When this amoeba is dividing it forms a large pseudopodium at each end (Fig. 17). This helps the newly forming individuals to pull apart. With their pseudopodia they move about, but remain connected for some time by a long slender filament. Finally one individual is stronger than the other, the connecting thread breaks and the two amoebae move apart, as free individuals. On the agar agar slides these newly formed individuals are easily recognized, especially when reconstruction is not entirely complete and the nuclei of the new individuals are in the same stage of reconstruction.

Multiple fission.

Multiple fission was occasionally observed on Petri dish cultures and one slide was made which contained over 350 amoebae in binary fission (Fig. 17), 44 which were dividing into three amoebae (Fig. 15), and many others which were dividing into four, five and even six (Fig. 16) new amoebae. All these dividing amoebae were large and contained numerous nuclei. There were also on this same slide other large multinucleate forms which had not begun to divide. It was a rich culture. The amoebae on the slide were so close together as to be crawling over each other for want of space and there were several masses of amoebae. There were literally thousands of amoebae on this one slide. Nuclear division had gone on more rapidly than the cytoplasmic division and this multiple fission was the means taken to get back to the normal uninucleate state. But even in this multiple fission the newly forming amoebae (Figs. 15, 16) have more than one nucleus so that it would take still another division to reach the normal condition.

This multiple fission has already been noted for limax amoebae by Wherry (1913), Walker and Sellards (1913), Cropper and Drew (1914) and others; and for *Entamoeba histolytica* recently by Yoshida (1920). However none of these observers have figured more than five new amoebae forming at one time.

Multiple nuclear division was found only once (Fig. 7). Here two nuclei side by side are beginning to form spindles in preparation for division. Later stages were not found. Multinucleate amoebae are usually formed by the repeated division of nuclei and not by the simultaneous division of two or more nuclei.

Budding.

Exogenous buds.

There are two kinds of buds, exogenous and endogenous. Both kinds of buds are found in a culture which is only two days old. Here the amoebae are getting an abundance of food, and are growing rapidly. There is much mitosis and many amoebae are dividing by binary fission, but there is still in each amoeba a great excess of chromatin material which has to be gotten rid of in some way. In these cultures the extra nuclei are seen crowded close up against the edge of the amoeba and even bulging out beyond it (Fig. 13*b*). This is the beginning of the formation of the first kind of exogenous bud.

The connection between this bud and the amoeba soon breaks and the bud floats off into the surrounding medium (Fig. 4). Some of the buds have quite a little cytoplasm cut off with them (Fig. 3), others have very little and these latter no doubt soon go to pieces. Those with the larger amount of cytoplasm grow and form some of the small amoebae which are so numerous in the older cultures.

In the cultures which are older, beginning with those which are six days old, a second kind of exogenous bud it found. The extra nuclei pass into the anterior ectoplasmic pseudopodium so that at times it is a solid mass of chromatin. Here they take a deep blue stain with iron haematoxylin (Fig. 8) and a deep red with safranin. They gradually work their way to the sides of the anterior pseudopodium and are then cut off as exogenous buds. Their position at the edge of the ectoplasm and at its junction with the endoplasm insure their getting both ectoplasm and endoplasm.

Endogenous buds.

In the two day old cultures there were numerous endogenous buds forming and quite a few which were already formed. Their nuclei were the extra nuclei which had been formed by the rapid nuclear division already noted in these young cultures.

The endogenous buds form within the endoplasm of the amoeba and are cut off from the main body and come to lie in a vacuole filled with clear liquid. This process is figured for *Vahlkampfia calkensi* (Hogue, 1914). First a crescent shaped vacuole appears and partially surrounds the bud which is forming. This vacuole increases in size and then completely surrounds the bud which is thus cut off from the amoeba and comes to lie in a vacuole within the amoeba. The newly formed bud frequently assumes amoeboid form (Fig. 12) and moves about within its vacuole by amoeboid motion. It gradually gets nearer to the surface of the amoeba (Fig. 2) and then the vacuole breaks to the outside, thus setting free the small amoeba.

Sometimes two amoebae were found in one sac (Fig. 5). It was not determined whether two amoebae were originally cut off into one sac or whether one bud divided into two amoebae. Probably the latter explantion is correct as buds with two nuclei (Fig. 9) were found in one vacuole.

Endogenous buds were found in cultures which were nine days old. After that there is little reproduction of this kind as the amoebae are then quite small. Endogenous buds are evidently another means of getting rid of the extra chromatin material as well as a means of reproduction.

The cycle of reproduction on a Petri dish.

In order to find out whether there was a definite sequence to these different methods of reproduction an experiment was performed in which slides were made every two days from one particular Petri dish culture. On the slides made when the culture was two days old, one noted the unusually large size of the nucleus compared to the amount of cytoplasm. This is one of the first signs that mitosis is going to take place. Here were many multinucleate forms, binary and multiple fission, much mitosis and quite a few endogenous buds, and many exogenous buds of the first type.

By the fourth day there were fewer binucleate and multinucleate forms. The amoebae were reproducing by binary fission and by endogenous buds and a few exogenous buds. On the sixth day the second kind of exogenous buds, those found in the anterior pseudopodia, appeared and were quite numerous. The amoebae were smaller, there was less binary fission and fewer endogenous buds were found. This condition continued until the ninth day. After that endogenous buds were not formed. Little fission was seen after this time though the exogenous buds continued to form along the sides of the amoebae and especially in the anterior pseudopodium. The experiment was discontinued after the fourteenth day as the amoebae were small and very few in number.

Cysts.

In many respects this amoeba is similar to *Vahlkampfia calkensi* which I found in oysters and have already described (Hogue, 1914). *Vahlkampfia calkensi,* however, had very characteristic cysts, of rather uniform size. They were three layers thick and dorsoventrally flattened. These cysts were practically always present in old cultures, where they were easily recognized by their irregular outline and dense appearance.

In the cultures of this amoeba, such cysts were never seen. This was the first thing to make me think I had a different amoeba. These amoebae, when the culture is old, disintegrate, leaving a plate full of débris with here and there a scattered amoeba still active and normal in its appearance. It is these older amoebae which occasionally encyst. As already stated, a Petri dish of .7 per cent. sodium chloride agar agar medium is completely covered with a dense growth of amoebae in a week's time. Cysts, however, do not begin to appear until the culture is at least ten days old, and then only a few cysts are scattered in between the active amoebae. Cysts were also formed when the amoebae were kept at 35° C.

The cysts are spherical (Fig. 14) and vary greatly in size. Those formed on Petri dishes which had been heated varied from 11–18 μ in diameter, those formed in the hanging drop in the thermostat varied from 4–12 μ in diameter. The cyst wall is thin, transparent and regular in outline. It must be slightly mucilaginous as cysts were found with particles adhering to them. Sometimes long streamers of small amoebae, bacteria and débris would remain attached to the cyst wall and wave back and forth in the currents under the cover slip. Inside, only the granular endoplasm is visible and it appears very dense and flocculent. The nucleus is distinct and easily seen. It is much more prominent here than in the active amoeba, where it appears only as a clear area, free from granules. The endoplasm frequently contains one or more vacuoles.

The cyst wall appears to be a semi-permeable membrane. No definite openings were found in it as Wilson (1916) describes for the soil amoeba. When neutral red or Donaldson's stain are added to the medium, the encysted amoebae soon take the red stain and in a short time go to pieces. For this reason it was found advisable not to use these stains which had been used by Kofoid (1919) and others to test the viability of the cysts. For another reason also the stains were not used. There are usually many active amoebae among the cysts. The addition of neutral red or Donaldson's stain made these amoebae round up and look like cysts, the clear ectoplasm distributing itself rather evenly around the spherical mass of endoplasm and giving the appearance of a cyst wall. These amoebae immediately took the stain and went to pieces more quickly than the stained cysts. They had evidently absorbed the water too quickly and then burst. They were often larger than the cysts and not always perfectly spherical. Occasionally they contained two nuclei while only one was ever found in the cysts. In the cysts too, the nucleus was more distinct than in the rounded amoebae.

After the amoeba had gone to pieces the nucleus remained intact for a long time and many of these nuclei would be found floating in the medium. These detached nuclei were present in all old cultures showing that the nuclear membrane is very tough and resistant.

With Donaldson's stain the vacuoles in the endoplasm sometimes stained pink, but never brown like the typical glycogen vacuoles of *Entamoeba histolytica, Entamoeba coli, Endolimax nana* and *Idoamoeba bütschlii.*

Flagellates.

In the early work with these amoebae before pure lines had been isolated, a small flagellate was frequently found with the amoebae in the cultures. I was anxious to see whether it was a stage in the life history of this amoeba. As soon as the pure lines were started, many attempts were made to induce enflagellation.

Schardinger (1899) was the first person to change amoebae into flagellates and then back again into amoebae. He says that temperature and food are the chief factors in the formation of flagellates. Repeating one of his experiments, with slight variations, amoebae were put in hanging drops of .7 per cent. sodium chloride solution on depression slides with hard vaseline rings. They were kept at 34.8° C. Hourly examinations were made one day and the following day they were repeatedly examined, but no flagellates appeared.

This experiment was repeated and the slides kept at 35° for a week. Then they were placed on the desk at room temperature, but no flagellates were formed. In one slide there were a few forms moving with flagellum like motion. However it was found that the .7 per cent. sodium chloride solution had not been sterile and there was the possibility of these forms being a contamination. When the experiment was repeated with freshly sterilized salt solution no flagellates were found.

Next, agar agar slants were made up with 1 and 1.5 per cent. sodium chloride solution. These were inoculated with the amoebae. It was hoped that the water of condensation in the bottom of the tube would induce the amoebae to enflagellate. When the amount of water of condensation was small, sterile .7 per cent. sodium chloride solution was added in small quantities to the test tubes. These slants and the water of condensation were examined for four months in smear preparations and in hanging drops, but flagellates were not found. Instead cysts were forming.

Again amoebae from six different pure lines were put into hanging drops of sterile .7 per cent. sodium chloride solution. These preparations were inverted over depression slides on parallel bars of hard vaseline so as to admit air. They were kept in moist chambers under frequent observation for forty-eight hours. No flagellates appeared. This experiment was repeated, but always with negative results.

Wherry (1913) has described a method by which he could practically always get flagellates from his cultures of soil amoebae. He grew the amoebae in hens' ovomucoid containing a trace of the egg yolk, and found that he could always obtain the flagellates if he took a loopful of the yolk ovomucoid and mixed it with 3 loopfuls of distilled water and placed it in a Barber moist chamber at 22–25° C. Accordingly hens' ovomucoid was made up with 1 and 1.5 per cent. NaCl solution. These tubes were inoculated with amoebae. Eight different pure lines were used. After the cultures were five days old a loopful was transferred to a cover slip and this was placed on parallel bars of hard vaseline over a depression slide in a moist chamber. The slides were under hourly observation for six hours and were again observed on the following morning, but flagellates did not at any time appear. These experiments were tried with many variations, in some cases .7 per cent. sodium chloride solution was added to the loop of yolk ovomucoid, but all efforts failed to give flagellates. The amoebae remained large and active, sending out many pseudopodia.

Still another attempt to obtain flagellates was made. Wilson (1916) says that the addition of distilled water to the medium was sufficient to cause the whole film of amoebae to enflagellate. Accordingly cover slips were prepared with one drop of Locke and one drop of sterile distilled water, also cover slips with one drop of .7 per cent. sodium chloride. Cover slips of Locke and of .7 per cent. sodium chloride solution were also made. These were all inoculated with amoebae taken from Petri dishes of Locke agar medium. Two different pure lines were used. These cover slips were inverted over depression slides on hard vaseline rings and kept in the thermostat at 35°. They were under hourly observation the first day and at frequent intervals on the following four days, but no flagellate forms appeared. The amoeba remained perfectly normal, moving about quite actively. These experiments were repeated the following week but with negative results.

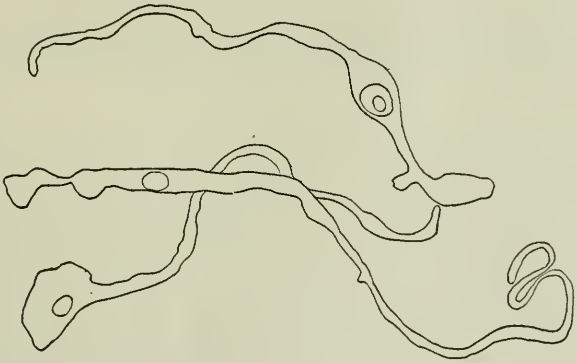
It seems altogether probable that this form of amoeba does not have a flagellate stage, since all attempts to procure flagellates have so far failed.

Age.

The amoebae seem to live indefinitely on Petri dishes of agar agar and .7 per cent. sodium chloride solution with .4 per cent. peptone. One of these cultures has been kept for over fourteen months and it still contains a few small active amoebae. A test tube slant culture of the same medium has active amoebae fifteen months after inoculation. In ovomucoid the amoebae remain large and active after fourteen months growth in this liquid. All these cultures were kept in moist chambers and at room temperature which varied from 18° C. in winter to the summer heat of Baltimore.

Effects of environment.

Media.—I have already shown (Hogue, 1917) that the density of the medium has a distinct effect on the shape of the amoebae. This was further demonstrated when the amoebae were grown on agar slides. If no moisture was added to the agar on the slide the amoebae would be found closely grouped together, fairly overlapping one another. When a loopful of sterile .7 per cent. sodium chloride solution was added to each slide the amoebae moved about freely and those at the edge were found in greatly elongated shapes (Text Fig. 1).



Text Figure 1. Greatly elongated amoebae grown on agar agar slides to which a drop of .7 per cent. sodium chloride solution has been added.

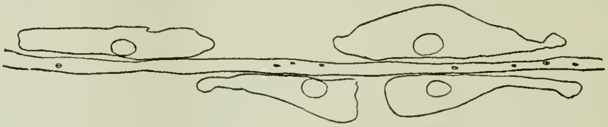
Uhlenhuth has already described (1915) this lengthening out of the cells as being due to the fact that the desired relation between the consistency of the medium and the consistency of the cell plasm has been reached resulting in greater freedom of movement. This difference in the shape of the amoeba has been noted for *Amoeba limax* by Thomson and Thomson (1916).

When neutral red was added to the .7 per cent. sodium chloride agar agar medium the amoebae did not flourish. They grew and in time some of the plates were completely covered with the amoebae, but it was not a luxuriant growth.

On the Locke agar medium the bacteria seem not to have gotten all the food necessary for their rapid growth. Consequently the amoebae multiply much more slowly on this medium than on that made up with sodium chloride, taking twice as long (two weeks at room temperature) to cover the Petri dish. At the end of this time there is an excellent growth. Their shape also is most fantastic, with many pseudopodia extended in search of food.

The amoebae planted on agar made up with distilled water and .4 per cent. peptone did not live. Even the bacteria often died and were never seen to spread over the agar. They were not getting the food necessary for their growth.

Another interesting effect of environment was occasionally seen on the slides which contained a drop of agar medium. Sometimes moulds would get started on these slides. The amoebae growing on the agar would come in contact with hyphae and immediately stretch out along these hyphae (Text Fig. 2). It reminded one of the shape of tissue culture cells when growing along the fibrils of a serum clot.



Text Figure 2. Amoebae slightly elongated, crawling along a mould hypha.

Liquid media.—Amoebae were planted in test tubes of sterile Locke solution. They lived here for two weeks but did not show any signs of multiplication by budding or by fission. Evidently the bacteria did not thrive and consequently the amoebae did not flourish. The experiment was not followed for longer than two weeks but it was then difficult to find the amoebae in the liquid. They were so small and so few in number.

In ovomucoid the amoebae have lived for over a year without being transplanted into a new medium. They are of excellent size, much larger than the average amoeba grown on .7 per cent. sodium chloride agar medium. The slides made from these cultures show amoebae of all sizes, multinucleate forms, binary fission, and those with endogenous buds. This is certainly one of the best media on which to keep the amoebae for long periods of time. However, they do not multiply as rapidly here as on the solid media. They are well nourished, of good size and nuclear division takes place as is evidenced by the multinucleate forms, but in order to have much fission this amoeba seems to need the contact with a solid medium.

Effects of temperature.

High temperature.—In order to test the effect of high temperature on the cultures a number of Petri dishes containing six different pure lines were put in a moist chamber and kept for twenty-four hours at 35° in the thermostat. The cultures were from two to eleven weeks old. When they were removed from the heat there were very few cysts found, but in a week's time all the cultures had numerous cysts.

Again amoebae from a four day old culture were put in a hanging drop of sterile .7 per cent. sodium chloride solution on a depression slide with a hard vaseline ring. These were kept at 35° C. forty-eight hours. At the end of this time many of the amoebae were rounding up and a few had encysted. There were still many active amoebae and quite a few of them had vacuoles. After 72 hours at 35° still more of the amoebae encysted, though others were still active. On a few slides the drop had spread out around the vaseline ring. On these slides the amoebae were all active and large and no cysts were found. These slides were kept for over a week at 35°. At the end of this time all the slides had some active amoëbae and most of them had cysts. The prevailing form was an amoeba, rounded up into a ball, quite inactive but of slightly irregular contour. Under low power it looked like a cyst but with higher magnification it appeared as an amoeba preparing to encyst.

Low temperature.—Several Petri dishes of four pure lines of amoebae were kept on the desk at room temperature, 18–23.5° C. A similar series were placed close to the window in December and January. Here the temperature ranged from 10–15° C. The amoebae divided more slowly in the cold though they were normal in size and shape. When the Petri dishes in the cold were not half covered with amoebae, those at room temperature would be completely covered. At room temperature you found amoebae of all sizes on one dish, while the corresponding culture in the cold had only amoebae of equal size, showing that they had not yet reached the stage of budding. This was due to the slowing down of the life processes at the low temperature. Again in these low temperatures three different pure lines of amoebae arranged themselves in compact groups over the Petri dishes, while at room temperature they were evenly distributed over the plate. Here their locomotion was affected by the cold.

Pathogenicity.

In order to test the pathogenicity of these amoebae it was decided to inoculate kittens with them. Accordingly five kittens were procured and daily microscopical examinations were made of their feces for 7, 7, 8, 8, and 11 days respectively. The feces of each kitten were also cultured on Locke agar. All examinations, both microscopical and cultural, showed no amoebae of any kind in the stools of the kittens.

Since it was very difficult to obtain cysts and since the cyst wall is quite permeable it was decided to inoculate the kittens per rectum. The amoebae from three Petri dishes were scraped off with a platinum loop and put into 3 cc. of sterile .7 per cent. sodium chloride solution. A drop of this was plated out on a Locke agar plate to be sure that the amoebae were in good condition when used for inoculation. All such transplants grew.

The kittens were anaesthetized and each one was inoculated per rectum with 3 cc. of the sodium chloride solution containing the amoebae. They were then given one-half grain of morphine hypodermically so as to slow down peristalsis.

After inoculation daily microscopical and cultural examination of the feces were made for all five kittens for 4, 7, 8, 8, and 11 days respectively. In no case were amoebae recovered. The kittens did not develop dysentery nor did they become infected with the amoebae. Walker and Sellards (1913) say that the amoebae fed in their experiments were recovered from 1–6 days after ingestion, not later, so that these examinations, where the inoculation was per rectum, should surely have shown the amoebae if they had been present in the rectum of the kittens. From these experiments one concludes that the amoebae are not infective to kittens and therefore probably not infective to man. In which case they are non-pathogenic forms.

Systematic position.

In naming this amoeba I am following Hartmann and Schilling's (1917) description for *Vahlkampfia* and putting it in that genus, which was first described by Chatton and Lalung-Bonnaire (1912). For a specific name I propose *patuxent* since the amoeba was found in the oysters from the Patuxent River, near Baltimore.

It can be seen from the description in the preceding pages that *Vahlkampfia patuxent* answers the original description of that genus given by Chatton and Lalung-Bonnaire. They say: "Le nouveau

genre *Vahlkampfia*, comprendra donc les amibes dont le noyau, constitué par un gros caryosome, avec ou sans centriole, peu de chromatine périphérique, se divise par promitose. Elles ont des kystes uninucléés."

Hartmann and Schilling (1917) give a somewhat fuller description, but with the same essential points. Doflein (1916), however, brings in the presence of a flagellate stage: "Besonders bemerkenswert ist ihre Fähigkeit in einen begeisselten freischwimmenden Zustand überzugehen." Neither Chatton and Lalung-Bonnaire (1912) nor Calkins (1912) nor Hartmann and Schilling (1917) include this stage in their description of Vahlkampfia. I therefore feel justified in placing this amoeba among the *Vahlkampfia,* since, so far, no flagellate stage has been observed in *Vahlkampfia patuxent.* Besides this, its nucleus has a large karyosome without a centriole, and with a small amount of peripheral chromatin. It divides by promitosis, and the cysts are uninucleate; all of which place it in the genus *Vahlkampfia.*

The preceding work is offered as a contribution to the life histories of parasitic amoebae. So far the life history of no parasitic amoebae is completely known. It is hoped that the present work on an amoeba parasitic in an invertebrate, the oyster, will be useful in the interpretation of some of the stages of development of amoebae parasitic in the higher vertebrates and especially of those parasitic in man.

Summary.

1. *Vahlkampfia patuxent* is a non-pathogenic parasite in the digestive tract of the oyster.
2. It has been cultivated in liquid media and on agar agar media made up with various salt solutions.
3. There is a distinct clear ectoplasm and a granular endoplasm.
4. The nucleus is eccentric with a large karyosome.
5. No contractile vacuole is present.
6. Division is promitotic.
7. Division into as many as six new amoebae is figured.
8. Exogenous and endogenous buds are formed.
9. Cysts are of rare occurrence. They are spherical and uninuclear. The cyst wall is thin and slightly mucilaginous.
10. A flagellate stage has not been observed, nor have any of the experiments performed produced flagellates.
11. The amoeba responded to the different media on which it was grown chiefly by a difference in the rate of growth.

12. Low temperature slowed down the rate of division.

13. High temperature caused the amoebae to round up and often to encyst.

14. Kittens inoculated per rectum with these amoebae, did not develop dysentery and were not infected with them.

15. The amoeba is not pathogenic to kittens and is therefore probably not pathogenic to man.

APPENDIX.

Formal description of *Vahlkampfia patuxent.*

Vahlkampfia patuxent has a clear ectoplasm and a granular endoplasm. There is usually a broad anterior pseudopodium, and several small slender posterior pseudopodia. It varies in size, but averages 21 μ in length and 13 μ in width. The nucleus is spherical with an eccentric spherical karyosome and a small amount of peripheral chromatin on the nuclear membrane. A grey area is visible, with certain stains, around the karyosome.

The nucleus divides by promitosis into two equal daughter nuclei, both the karyosome and peripheral chromatin taking part in the process. Binary and multiple division takes place. There is no contractile vacuole. Endogenous and exogenous buds are formed. The cysts are spherical, 11–18 μ in diameter. The cyst wall is thin, transparent and regular in outline, without openings. No flagellate stage has been found.

It is very similar to *Vahlkampfia calkensi* (Hogue, 1914), but differs from it in having a very simple cyst wall.

Its nuclear phenomena in division are very similar to many of those given by Wilson (1916) for *Naegleria gruberi*, but *Vahlkampfia patuxent* differs from it in being a salt water form without a contractile vacuole and in not having a flagellate stage.

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EXPLANATION OF PLATES.

All the figures of *Vahlkampfia patuxent* were drawn with a camera lucida. The plates have been reduced one third.

Plate I.

The drawings were made with 1.9 mm. objective and 7.5 ocular.

Fig. 1. A characteristic form showing broad anterior ectoplasmic pseudopodium and a few slender posterior pseudopodia. The nucleus contains an eccentric karyosome.

Fig. 2. Amoeba with one endogenous bud just ready to break through the thin wall of its vacuole. There are two exogenous buds. The nucleus with its eccentric karyosome shows the peripheral chromatin condensed on one side of the nuclear membrane.

Fig. 3. An exogenous bud being cut off. Mann's stain was used, which brings out the grey area around the karyosome.

Fig. 4. Extra-nuclear bud which has just been given off.

Fig. 5. Amoeba with two endogenous buds in one vacuole. The nucleus shows the peripheral chromatin condensed in plates on the nuclear membrane.

Fig. 6. Giant amoeba with six nuclei. The cytoplasm is full of vacuoles, many containing granular material, which in one vacuole is dividing as the vacuole is constricted by the movement of the amoeba.

Fig. 7. Binucleate amoeba with both nuclei in the early prophase of promitosis.

Fig. 8. Binucleate amoebae with the extra chromatin condensed in the large anterior ectoplasmic pseudopodium. A small exogenous bud is forming at the side.

Fig. 9. Amoeba with a binucleate endogenous bud.

Fig. 10. Amoeba with one endogenous bud formed and eleven exogenous buds forming along the edge.

Fig. 11. Dividing amoeba filled with large vacuoles. The nuclei are abnormal, being broken up into four and five small parts.

Fig. 12. Amoeba with a large endogenous bud which is already moving with amoeboid motion in its vacuole.

Fig. 13*a*. Multinucleate amoeba preparing to divide. Two nuclei are in the process of reconstruction. The equatorial plate chromatin has not yet fused with the chromatin masses.

Plate II.

Fig. 13*b*. A multinucleate amoeba with one exogenous bud cut off and a nucleus near the edge ready to form a second bud. 7.5 ocular, 1.9 mm. objective.

Fig. 14. A cyst showing the prominent nucleus and the endoplasm filled with large refractile granules. 12.5 ocular and 1.9 mm. objective.

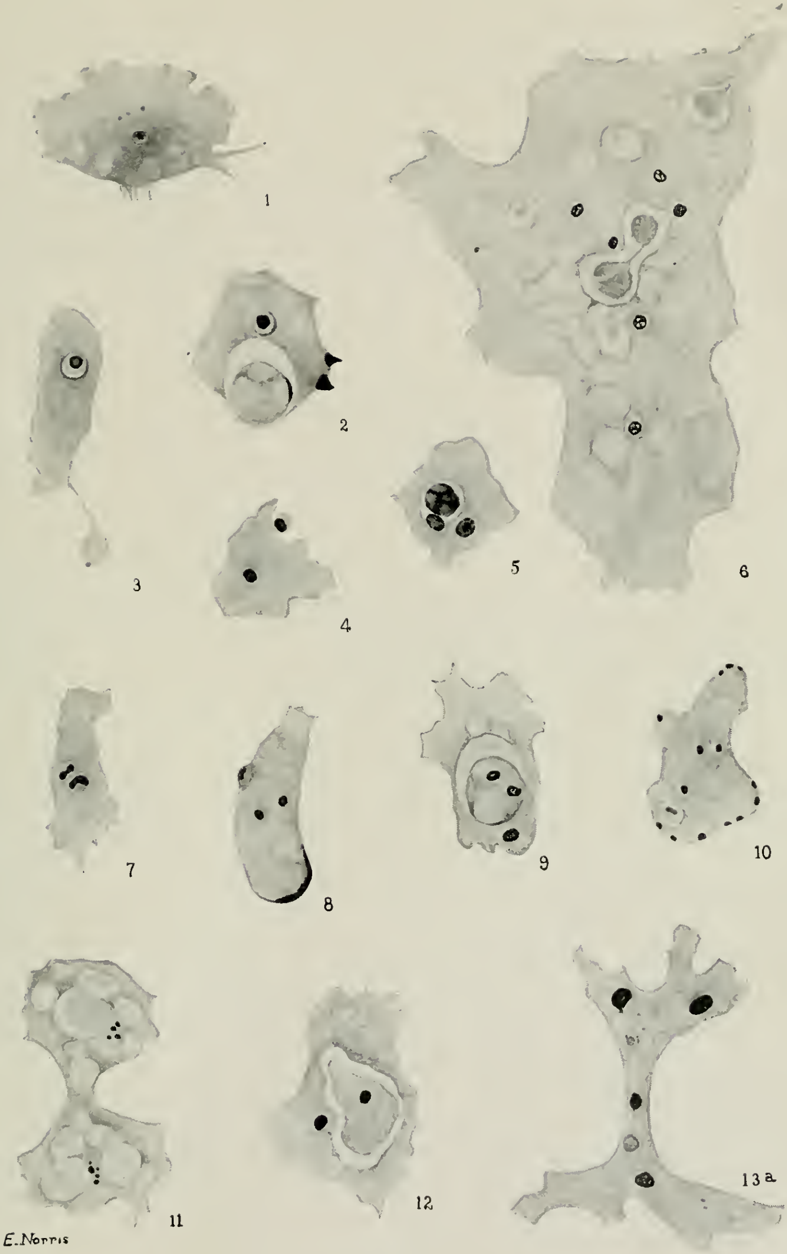


PLATE IX.

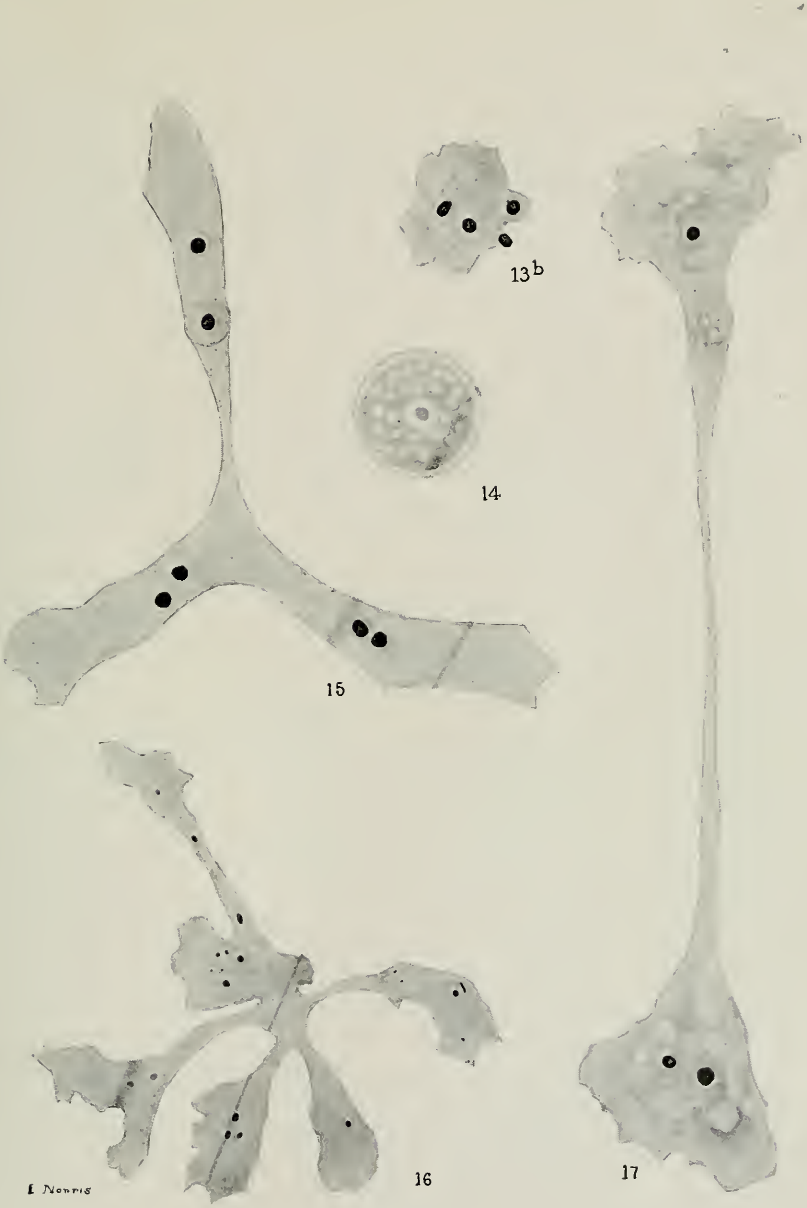
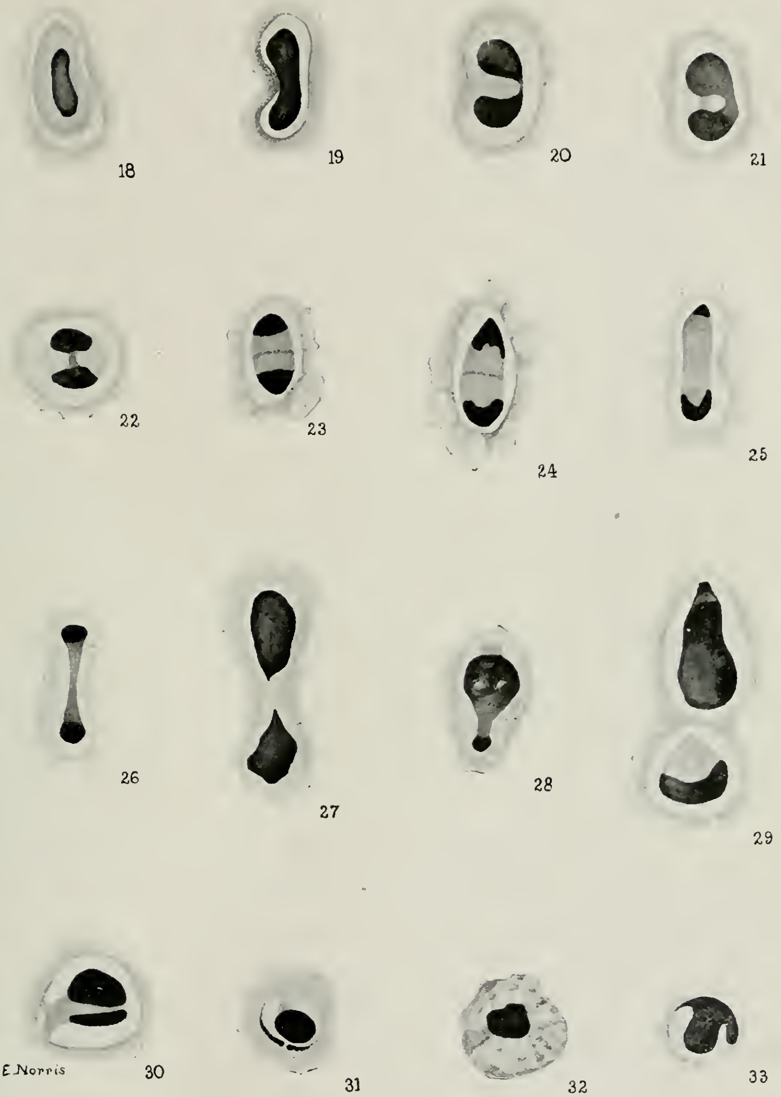


PLATE X.



E. Norris

PLATE XI.

Fig. 15. A multinucleate amoeba dividing to form two binucleate amoebae and one amoeba with an endogenous bud. 7.5 ocular and 1.9 mm. objective.

Fig. 16. A multinucleate amoeba dividing to form six new amoebae. 5 ocular, 1.9 mm. objective.

Fig. 17. Amoebae dividing to form two amoebae. 7.5 ocular, 1.9 mm. objective.

Plate III.

All drawings made with 18 ocular and 1.9 mm. objective.

Fig. 18 Prophase: Karyosome, grey area and nuclear membrane all elongating.

Fig. 19. Elongated karyosome being indented by the accumulation of peripheral chromatin at one side.

Fig. 20. Peripheral chromatin dividing the karyosome into the two chromatin masses.

Fig. 21. The spindle fibres showing as a strand between the chromatin masses.

Fig. 22. Formation of the spindle between the chromatin masses which are still connected (front view).

Figs. 23, 24. Late prophase showing broad chromatin masses and equatorial plate.

Fig. 25. Anaphase constriction of the nuclear membrane. Dark areas near the chromatin masses, probably the chromatin from the equatorial plate.

Fig. 26. Late anaphase.

Fig. 27. Telophase. The spindle has divided and the chromatin is distributing itself on the spindle fibers.

Figs. 28–33. Reconstruction.

Fig. 28. Nucleus showing clear polar cap at one end, the chromatin mass and peripheral chromatin connected by the spindle fibers. The clear spaces are plastin.

Fig. 29. Two phases in reconstruction.

Figs. 30, 31, 32, 33. Chromatin material pouring out onto the nuclear membrane where it is condensing.

A STUDY OF THE NATURAL AND ACQUIRED ANTISHEEP HEMOLYSINS OF THE RABBIT AS REGARDS THERMOLABILITY.*

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The so-called natural antibodies found in the sera of many animal species have attracted attention from various points of view, viz., their origin, specificity, relation to natural immunity and particularly their relation to the corresponding acquired antibodies. The point mentioned last is of especial significance in connection with the origin of acquired antibodies and has, therefore, been the subject of a number of investigations. The view is held by some investigators (1) (2) that the identity of these two classes of antibodies has been established, while others (3) do not accept the evidence which has been advanced in favor of their identity, or have pointed out instances wherein a natural antibody is qualitatively different from the corresponding acquired antibody. Thiele and Embleton (4) have recently advanced a theory of the origin of acquired hemolysins which involves the existence of thermolabile and thermostabile hemolysins. Sherman (5) questions the soundness of this theory because he found all hemolysins to be thermostabile. Kolmer (6), on the other hand, concludes that natural hemolysins are less resistant to heat than immune hemolysins.

The purpose of the present paper is to record observations which we have made concerning the effects of different degrees of heat on the natural and immune hemolysins of the rabbit for sheep corpuscles.

Experimental.

Four rabbits (two normal and two immune) were selected for the study on the basis of the hemolytic titers of their sera, a wide range of titer being sought. The serum of one of the normal rabbits had a titer 1–10, the other 1–100; while the serum of one of the immunized rabbits had a titer of 1–2,500 and the other 1–25,000. The point to be determined was the rate of loss of amboceptor content of the different sera heated at different temperatures.

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Technique.—The corpuscles used in the titrations were collected from the sheep in a solution of sodium citrate and then washed four times in large volumes of salt solution (0.85 per cent. NaCl) and resuspended in salt solution so that the corpuscles were one fourth as concentrated as in the original blood. One tenth of a cubic centimeter of this suspension was taken as a unit of cells. Pooled guinea pig serum was diluted with four parts of salt solution and 0.1 cc. of the diluted serum was used as a unit of complement. The sera to be titrated were heated in a water bath for thirty minutes at varying degrees of temperature (ranging from 56° C. to 84° C.) and then diluted with salt solution in order that graded quantities could be conveniently put in a series of serological tubes. Salt solution was now added to make the volume in each tube 0.8 cc. A unit of cells was then put in each tube and the tubes were placed in a water bath (temperature 37° C.) for one hour. At the end of the incubation period a unit of complement was added and the tubes were returned to the water bath for another hour. The extent of hemolysis was observed at the expiration of the second incubation period and again after the tubes had stood at room temperature for twelve hours. Different degrees of hemolysis are indicated in the protocols by numerals; 4 indicates complete hemolysis, 0 no hemolysis and the intervening figures a corresponding degree of hemolysis. Protocol 1 illustrates the type of titration experiments used throughout the work.

PROTOCOL 1.

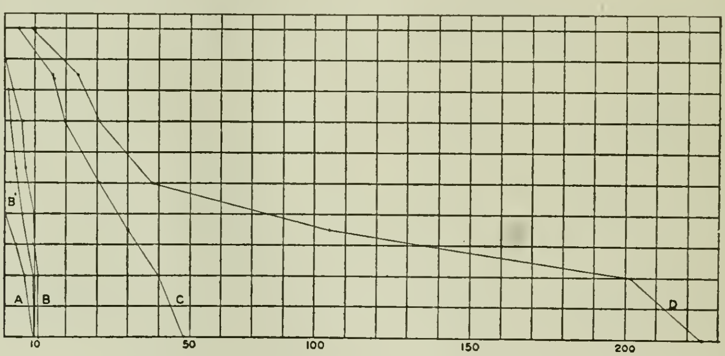
Type protocol used in the titration experiments.

Tubes.....	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	(1–0)*				(1–10)					(1–100)					
Serum...	0.6	0.4	0.2	0.1	0.6	0.4	0.3	0.2	0.1	0.8	0.6	0.4	0.2	0.0	0.0
Salt.....	0.2	0.4	0.6	0.7	0.2	0.4	0.5	0.6	0.7	0.0	0.2	0.4	0.6	0.8	0.9
Cells 1–4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Incubated 1 hour at 37° C.															
Complement 1–5	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0

* The numbers in parenthesis indicate the extent to which the serum was diluted before the different quantities were measured into the various tubes. Thus tubes 1 to 4 contained the indicated quantities of undiluted serum and tubes 5 to 9 serum diluted 1–10 with salt solution, etc.

The effect of different temperatures on immune hemolysins.—The question here was the determination of the rate at which the immune amboceptor disappears from the serum when subjected to heat for 30 minutes through a graded series of temperatures. The two immune sera which have already been mentioned were used for this purpose. The sera are further described under sections (*a*) and (*b*), and the results of the experiment are recorded in Tables I and II respectively.

(*a*) *Antisheep rabbit 266.*—The serum from this rabbit was very potent, the titer being 1–26,000 after inactivation at 56° C. for 30 minutes. Specimens of the serum were kept at six different degrees of temperature (56° C.–84° C.) for 30 minutes and then titrated for hemolytic power. The results are collected in Table I and are represented graphically by Curve *D* in the text figure.



These curves are based on "1" degree of hemolysis at the 12-hour readings. The temperatures at which the sera were heated are expressed by the ordinates, while the abscissae express the square roots of the hemolytic titers. The curves show the rate of destruction of the hemolysins when heated through a range of temperatures above 56°C. Curve *A* represents normal rabbit serum, low titer (Table III); Curve *B*, normal rabbit serum, high titer (Table IV); Curve B,^{1} immune hemolysins in normal rabbit serum (Table V); Curve *C*, immune serum, low titer (Table II); Curve *D*, immune serum, high titer (Table I).

(*b*) *Antisheep rabbit 260.*—The serum from this rabbit was about one tenth as potent as the serum from rabbit 266. The results obtained with this serum are given in Table II and Curve *C* (*see text figure*).

Curves *C* and *D* obtained with the two immune sera of widely different titers are directly comparable. It is seen that the curves are parallel beyond 66° C. and that the hemolysins practically disappear at 76° C. Between 60° C. and 66° C. there is a marked difference showing that the rate of destruction is greatest between these two temperatures for the serum of the higher titer.

TABLE I.

The effect of different temperatures on immune serum, rabbit 266.

Temperature of inactivation.	Titer of the serum at 1 hour.	Titer of the serum at 12 hours.
56° C.	35,000†	50,000
60° C.	30,000	41,000
63° C.	8,000	11,000
66° C.	1,700	2,300
70° C. (1–2)*	730	1,015
73° C. (1–2)	400	600
76° C. (1–2)	50	70
84° C. (1–4)	6	0

TABLE II.

The effect of different temperatures on immune serum, rabbit 260.

Temperature of inactivation.	Titer of the serum at 1 hour.	Titer of the serum at 12 hours.
Unheated	5,000†	6,700
56° C.	3,300	3,300
60° C.	2,200	2,500
63° C.	1,540	1,666
66° C.	1,000	1,000
70° C. (1–2)*	333	400
73° C. (1–2)	250	260
76° C. (1–2)	10	20
80° C. (1–4)	8	13

The effect of different temperatures on natural hemolysins.

(*a*) *Normal rabbit serum, low titer.*—After inactivation at 56° C. this serum failed to cause complete hemolysis in dilution above 1–10. The effect of higher degrees of temperature on this serum is shown in Table II. It is seen that the hemolysins were completely destroyed at 64° C. Curve *A* (see text figure) gives a graphic representation of the rate at which the hemolysins were destroyed.

(*b*) *Normal rabbit serum high titer.*—This serum caused complete hemolysis in a dilution of 1–50 after inactivation at 56° C. The effects of higher temperatures are shown in Table IV. It is seen that a temperature of 70° C. does not completely destroy the hemolysins of this serum (see Curve *B*).

A study of the foregoing tables shows that all the sera, both normal and immune, contained thermostabile hemolysins, i.e., hemolysins that were not destroyed by being heated for 30 minutes at 56° C. It is

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* The figures in parentheses indicate the dilution of the serum before heating.

† These numbers represent the number of hemolytic units per cc. of serum.

TABLE III.

The effect of different temperatures on natural hemolysins, serum of low titer.

Tubes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Serum	(1–0)* 0.6	0.4	0.3	0.1	(1–10) 0.6	0.5	0.4	0.3	0.2	0.1	(1–100) 0.6	0.5	0.4	0.3	0.2	0
1 hour reading:																
Active, no complement	4	4	4	1−	0	0	0	0	0	0	0	0	0	0	0	0
Unheated	4	4	4	4	1	1−	1−	1−	0	0	0	0	0	0	0	0
56° C.†	4	4	4	4	1	1−	1−	1−	1−	0	0	0	0	0	0	0
60° C.	3	3	3	3	1−	1−	1−	1−	0	0	0	0	0	0	0	0
62° C.	3	3	3	1	1−	1−	0	0	0	0	0	0	0	0	0	0
64° C.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 hour reading:																
Active, no complement	4	4	4	2	1−	0	0	0	0	0	0	0	0	0	0	0
Unheated	4	4	4	4	2	2	2	2	1	1	1−	0	0	0	0	0
56° C.	4	4	4	4	2	2	2	2	1	1	1−	0	0	0	0	0
60° C.	3	3	3	3	3	3	3	1	1	1−	0	0	0	0	0	0
62° C.	3	3	3	3	1	0	0	0	0	0	0	0	0	0	0	0
64° C.	1−	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* The figures in parentheses indicate the extent to which the serum was diluted before the quantities indicated were added to the tubes. Thus, tubes 1–4 received undiluted serum and tubes 5–10 received serum diluted 1–10 with salt solution, etc.

† The heated serum was reactivated with a unit of complement, 0.1 cc. of guinea pig serum diluted 1–5 with salt solution.

TABLE IV.

The effect of different temperatures on natural hemolysins, serum of high titer.

Tubes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Serum	(1–0) 0.6	0.4	0.2	0.1	(1–10) 0.6	0.4	0 3	0.2	0.1	(1–100) 0.8	0.6	0.4	0.2	0
1 hour reading:														
Active, no complement	4	4	4	4	4	3	3	1–	1–	0	0	0	0	0
Unheated	4	4	4	4	4	4	4	1–	1–	0	0	0	0	0
56° C.	4	4	4	4	4	4	4	1–	1–	0	0	0	0	0
60° C.	4	4	4	4	4	4	3	3	0	0	0	0	0	0
62° C.	3	3	3	4	4	4	3	2	0	0	0	0	0	0
64° C.	3	3	3	4	4	4	4	1	0	0	0	0	0	0
67° C.	4	4	4	4	4	3	1	0	0	0	0	0	0	0
70° C. (1–2)	3	3	3	3	2	1–	0	0	0	0	0	0	0	0
72° C. (1–2)	1	1	1	1	0	0	0	0	0	0	0	0	0	0
12 hour reading:														
Active, no complement	4	4	4	4	4	3	3	1–	1–	0	0	0	0	0
Unheated	4	4	4	4	4	4	4	4	3	2	1–	0	0	0
56 C.	4	4	4	4	4	4	3	3	3	1	1–	0	0	0
60° C.	4	4	4	4	4	4	4	4	2	1	1–	0	0	0
62° C.	3	3	3	4	4	4	4	3	1	1–	0	0	0	0
64° C.	3	3	3	4	4	4	4	3	1	1–	0	0	0	0
67° C.	4	4	4	4	4	4	3	3	1–	1–	0	0	0	0
70° C. (1–2)	4	4	4	4	4	4	4	2	1–	0	0	0	0	0
72° C. (1–2)	1	1	1	1	0	0	0	0	0	0	0	0	0	0

seen that in one of the normal sera (the one of low titer, Curve *A* the hemolysins were completely destroyed by a temperature of 64° C., while complete destruction of the hemolysins was not effected in the other normal serum (the one of high titer, Curve *B*) until a temperature of 74° C. was reached. The two immune sera, being of widely different titers, show that the number of units of hemolysin destroyed within certain temperature limits (60° C.–66° C.) depends upon the concentration of the antibody in the serum (Curves *C* and *D*), but that in each case a certain residue of antibody remains which requires practically the same amount of heating to destroy completely. A comparison of the four curves indicates that the thermal death point of the sera (complete destruction of the hemolysins) is a function of the hemolytic potency of the sera rather than the kind (natural or immune) of hemolysin present. This point will be referred to again.

The effect of heat on diluted immune hemolysins.—The foregoing experiments having indicated that the degree of heat necessary to effect complete destruction of the hemolysins in a given serum depends upon the concentration rather than the kind (natural or immune) of hemolysin present, it was desirable to test this point further by testing the effect of heat on two sera of the same potency, one containing natural hemolysins and the other immune hemolysins. For this purpose an artificial immune serum was prepared in such a way that it would have the same titer as a given normal serum. The normal serum, which has already been discussed (Table IV), contained 100 hemolytic units per cc. The immune serum was prepared by two methods and designated Solutions *A* and *B* respectively.

Solution A.—Normal rabbit serum was freshly collected and rendered free of hemolysins by adsorption at 4° C. with an excess of washed sheep corpuscles. The corpuscles were removed from the serum by centrifugalization and the desired amount of a highly potent immune serum was added. The artificial serum had a titer of 1–100 after inactivation at 56° C. for 30 minutes.

Solution B.—This preparation was the same as Solution *A* with the exception that salt solution instead of normal rabbit serum was used to dilute—the immune serum. The titer was the same as that of Solution *A*.

The effect of different degrees of heat on the hemolytic titers of both of these solutions was determined, the plan of the experiment being the same as in previous experiments. The results are given in Tables V and VI respectively.

TABLE V.

The effect of different temperatures on immune hemolysins, artificial serum A.

Tubes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Solution A	(1–0) 0.6	0.4	0.2	0.1	(1–10) 0.6	0.4	0.3	0.2	0.1	(1–100) 0.8	0.6	0.4	0.2	0
1 hour reading:														
Adsorbed control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unheated	4	4	4	4	4	4	4	2	2	1–	0	0	0	0
56° C.	3	2	2	2	1–	1–	1–	1–	0	0	0	0	0	0
60° C.	3	3	3	3	1–	0	0	0	0	0	0	0	0	0
62° C.	4	4	4	3	1–	0	0	0	0	0	0	0	0	0
64° C.	3	3	3	1–	1–	0	0	0	0	0	0	0	0	0
67° C.	3	3	1	1–	1–	0	0	0	0	0	0	0	0	0
70° C. (1–2)	4	3	0	0	0	0	0	0	0	0	0	0	0	0
74° C. (1–2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 hour reading:														
Adsorbed control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unheated	4	4	4	4	4	4	4	3	3	2	1–	0	0	0
56° C.	3	3	3	4	3	3	2	1	1–	1–	0	0	0	0
60° C.	4	4	4	4	3	3	3	2	1–	1–	0	0	0	0
62° C.	4	4	4	4	3	1	1–	0	0	0	0	0	0	0
64° C.	3	3	3	4	4	1	0	0	0	0	0	0	0	0
67° C.	4	4	3	2	1	1–	0	0	0	0	0	0	0	0
70° C. (1–2)	4	4	1	0	0	0	0	0	0	0	0	0	0	0
74° C. (1–2)	1	1–	0	0	0	0	0	0	0	0	0	0	0	0

TABLE VI.

The effect of different temperatures on immune hemolysins, artificial serum B.

Tubes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Solution B	(1–0) 0.6	0.4	0.2	0.1	(1–10) 0.6	0.4	0.3	0.2	0.1	1–100 0.8	0.6	0.4	0.2	0
1 hour reading:														
56° C.	4	4	4	4	4	4	4	3	1–	0	0	0	0	0
60° C.	4	4	4	4	4	4	4	3	1–	0	0	0	0	0
62° C.	3	3	4	4	4	3	1	1	0	0	0	0	0	0
64° C.	3	3	3	3	3	1	0	0	0	0	0	0	0	0
67° C.	3	3	4	4	1–	0	0	0	0	0	0	0	0	0
70° C. (1–2)	4	4	1–	0	0	0	0	0	0	0	0	0	0	0
74° C. (1–2)	4	1–	0	0	0	0	0	0	0	0	0	0	0	0
12 hour reading:														
56° C.	4	4	4	4	4	4	3	3	1	1–	0	0	0	0
60° C.	4	4	4	4	4	4	4	3	2	1	0	0	0	0
62° C.	4	4	4	4	4	4	3	2	1–	0	0	0	0	0
64° C.	3	3	3	4	4	1	1	1	0	0	0	0	0	0
67° C.	4	4	4	4	4	1	1–	0	0	0	0	0	0	0
70° C. (1–2)	4	4	4	1	1–	0	0	0	0	0	0	0	0	0
74° C. (1–2)	4	4	1	0	0	0	0	0	0	0	0	0	0	0

It is seen from Tables V and VI that immune hemolysins diluted with salt solution are only slightly more susceptible to the action of heat than when diluted with normal serum. It is of particular interest to note that the normal hemolytic serum (Table IV) and the artificial immune serum resist destruction by heat through practically the same range of temperatures (compare Curves *B* and *B′* in the text figure). It is further indicated therefore, that the rate at which hemolysins are destroyed by heat, as well as the temperature necessary to destroy completely the amboceptor content of a given serum, depends upon the quantity rather than the quality of the hemolysins.

TABLE VII.

The effect of salt solution on the thermolability of immune hemolysins.

Diluent.	Temperature.	1 hour, 4.	12 hours, 4.
Salt	56° C.	25,000	33,000
Serum	56° C.	25,000	33,000
Salt	60° C.	25,000	25,000
Serum	60° C.	25,000	25,000
Salt	63° C.	8,333	8,333
Serum	63° C.	8,333	8,333
Salt	66° C.	625	625
Serum	66° C.	625	625
Salt	70 C.	500	500
Serum	70° C.	500	500

The relative thermolability of hemolysins in the presence and absence of other serum constituents.—In the foregoing experiments, as will have been seen, it was necessary to dilute the hemolytic sera before testing the effects of the higher temperatures on them because undiluted serum forms a firm coagulum at these temperatures. Physiological salt solution was used as the diluent. The propriety of this procedure was questioned at the time. A casual experiment showed, however, that there was no great difference in the heat susceptibility of hemolysins when diluted with salt solution and when in undiluted serum. Since this result was not in agreement with what one would be inclined to conclude *a priori* and since the conclusions to be drawn from other experiments (see Table VI) are based upon the assumption that dilution with salt solution does not affect the thermolability of immune hemolysins, it was deemed advisable to subject this point to more exact tests.

An immune antisheep rabbit serum of very high titer was chosen for this experiment. Two preparations were made with the immune serum: (1) 1 cc. of the serum was added to 49 cc. of salt solution; (2) 1 cc. of the hemolytic serum was added to 49 cc. of normal rabbit serum from which the natural hemolysins had been removed by specific adsorption. The two preparations were heated in 3 cc. quantities at different temperatures for 30 minutes and the hemolytic titers determined. The results are given in Table VII.

Table VII shows that the thermolability of hemolysins is in no way affected by the presence or absence of other serum constituents. The first preparation contained 98 per cent. of salt solution during the heating process, while in the second preparation the salt solution was replaced by normal rabbit serum. The hemolytic titers of the preparations were exactly the same.

Discussion.

The results of the experiments recorded in this paper seem to support the contention that natural hemolysins are qualitatively the same as the corresponding acquired or immune hemolysins. It has been shown that two specimens of normal serum containing different quantities of hemolysins for a particular corpuscle will, on being heated at different temperatures, give the impression that the antibodies of one specimen are more sensitive to heat than those of the other. However, the serum containing the more sensitive antibodies is found to be the one which had the lowest original titer. It would follow, therefore, that a serum of very low titer might be entirely inactive (not contain an appreciable amount of antibody) after inactivation at 56° C. for 30 minutes, and thus give rise to the impression that only thermolabile antibodies were present, while a more potent serum of the same kind would give the opposite result. A consideration of normal sera alone leads to the conclusion that the amount of heat necessary permanently to inactivate a serum depends in part upon the quantity of antibody present.

In comparing normal and immune sera, it was found that natural and acquired hemolysins are equally resistant to heat. The comparison was made possible by obtaining normal and immune sera of the same potency. The immune serum was prepared artificially, but it was shown by experiment that its sensitivity to heat was not thus affected. It seems fair to conclude, therefore, that the apparent relative thermolability of the natural hemolysins, as compared with acquired hemolysins, is due to a quantitative rather than a qualitative difference.

SUMMARY.

1. The natural hemolysins of the rabbit for sheep corpuscles are as heat resistant as the corresponding immune hemolysins.

2. The apparent difference in the thermolability of natural and immune hemolysins is due to the relative potency of the sera, the more potent serum apparently being more resistant to heat.

3. Hemolysins are just as resistant to heat when in salt solution as in undiluted serum.

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THE REACTIVATION OF THE NATURAL HEMOLYTIC ANTIBODY IN CHICKEN SERUM.*

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The fresh blood serum from many animal species hemolyzes the red cells of other species. In general the reaction has been shown to be due to a sensitizer-alexin complex, the natural sensitizers (hemolysins) being very similar to the specific immune hemolysins. For example, they resist heating for 30 minutes at 56° C., they sensitize red blood corpuscles to the action of alexin from other species, and they can be removed from the serum by adsorption with the red cells for which they are hemolytic. The natural hemolysins of the domestic fowl are, however, peculiar in that it has been found difficult or impossible to reactivate the serum with the fresh sera of other species after it has been rendered inactive by heat. In fact the presence of a heat resistant antibody has been questioned.

Bordet (1) was the first to observe the hemolytic properties of chicken serum. He called attention to the marked susceptibility of rabbit corpuscles to the hemolytic and agglutinating action of this serum. Metchnikoff (2) states that Bordet found it impossible to reactivate chicken serum with any other serum, after the former had been heated for 30 minutes at 55–56° C., and that he (Bordet) was of the opinion that in this instance alexin alone was capable of producing hemolysis, previous sensitization with an immune body being unnecessary. We have been unable to find the above statement in Bordet's own publication, but in discussing the relation between hemagglutination and the sensitization of red blood corpuscles Bordet (1) does state that rabbit red blood corpuscles, although strongly agglutinated by heated chicken serum, are no more susceptible to the slight dissolving effect of normal guinea-pig serum than are normal rabbit corpuscles.

P. Müller (3) verified Bordet's observation, but concluded that the hemolytic action of chicken serum is to be ascribed to an amboceptor-alexin complex, and that the difficulty experienced in reactivating the serum arises from the fact that the amboceptors of chicken serum have complementophile groups which do not react with the haptophore groups of complements from other species. Müller claims to have established this point by showing that inactivated chicken serum can be reactivated with fresh serum from treated chickens and with the serum from the pigeon, a closely related species, but not with serum from other species. Müller's experiments on this point were briefly as follows: Chickens were given intraperitoneal injections (daily for from 1 to 3 days) of bouillon, peptone water or suspensions of aleuronat, and were bled from 12 hours to 3 days after the last injection. The serum from this blood was used as complement, the supposition being that the treatment had increased the complement content of the serum. The serum from untreated chickens was inactivated by heat and used as amboceptor. Müller's protocols show that from 0.05 cc. to 0.07 cc. of the complementing serum plus 0.2 cc. of the heated serum hemolyzed a unit of cells, and that 0.1 cc. of the complementing serum alone also hemolyzed a unit of cells. The protocols on reactivation with pigeon serum show that 0.15 cc. of fresh pigeon serum hemolyzed a unit of rabbit cells with 0.2 cc. of inactivated chicken serum as amboceptor. The hemolytic dose of the pigeon serum alone was about 0.3 cc.

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The object of the present paper is to present new observations on the reactivation of the natural hemolysins of chicken serum for rabbit red cells complemented by the fresh sera of the chicken and the guinea pig after inactivation by heat.

Experimental.

General Technique.—The serum used in the following tests was freshly collected and separated from the clot by centrifugalization. The sensitizing serum, as well as the serum used for complement, was pooled from two or more animals. The red corpuscles were freshly collected in citrate, washed four times in large volumes of 0.85 per cent. salt solution and resuspended in salt solution to a dilution of 1–4 in terms of whole blood. One tenth of a cubic centimeter of this suspension was the unit of cells, while the complements were diluted so that the required dosage was contained in 0.1 cc. The reagents were added at room temperature to small serological tubes and incubated at 37° C. Readings were made after one hour's incubation and are recorded in the table as 4 + complete hemolysis; ±slight hemolysis and 0, no hemolysis.

Hemolytic titer of fresh chicken serum for rabbit red cells.—The hemolytic action of fresh chicken serum for rabbit red cells was determined according to Protocol 1. The first five figures in this protocol following the heading "Serum" represent the indicated amounts of undiluted serum added to the different tubes. The sixth and following tubes received the serum previously diluted 1–16 with salt solution.

PROTOCOL 1.

Fresh chicken serum vs. rabbit corpuscles.

Tubes....	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Serum....	(1–0) 0.6	0.4	0.3	0.2	0.1	(1–16) 0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.05	0
Serum......	(1–0) 0.6	0.4	0.3	0.2	0.1	(1–16) 0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.05	0
Salt solution	0.3	0.5	0.6	0.7	0.8	0.1	0.3	0.4	0.5	0.6	0.7	0.6	0.85	0.9
1–4 corpuscles...	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1 hour reading.......	4+	4+	4+	4+	4+	+	±	±	0	0	0	0	0	0

Many titrations carried out in the same way have consistently shown that 0.1 cc. of fresh chicken serum completely dissolves the dose of rabbit cells used here. In some cases, however, in the higher dilutions more or less marked hemolysis appears after one hour's incubation. In exceptional cases slight hemolysis has appeared after one hour's incubation in doses as small as 0.025 cc., with complete hemolysis in doses of 0.05 cc. Determinations made in the same way for cells of the guinea pig, ox, sheep and pigeon have shown that fresh chicken serum is hemolytic for these cells. Under the conditions of the experiment, however, the dissolving effect of the fresh serum is most energetic toward rabbit cells and least for those of the pigeon.

The reactivation of chicken serum, ordinary method.—Hemolytic tests were made with heated chicken serum and the red cells of the rabbit, in which the fresh serum of the guinea pig and chicken were used as complements in doses of 0.025 cc. Fresh chicken serum was heated to 56° C. for 30 minutes. The heated serum was added to tubes as in Protocol 1, with 0.1 cc. less of salt solution which was replaced with the same volume of complement serum diluted to give the required dosage. Complement and cells were added as in an ordinary hemolytic titration. The tubes were thoroughly shaken and then incubated. No hemolysis was evident at the end of one hour. Tests were also made in which the heated serum, salt solution and cells were incubated one hour before the complement was added. The tubes were then thoroughly shaken and incubated for one hour longer. There was no hemolysis in this case.

So far, it is seen that fresh chicken serum actively hemolyzes foreign red blood corpuscles, particularly those of the rabbit and that the inactivated serum is not reactivated with fresh sera in ordinary hemolytic tests.

The reactivation of chicken serum, new method.—By a modification of the original technique, chicken serum inactivated by heat at 56° C. for 30 minutes was reactivated for rabbit corpuscles with non-hemolytic doses of fresh chicken serum to practically its original hemolytic titer. The test was made by adding the different amounts of heated chicken serum to tubes as indicated under the tube numbers in the table. Salt solution was then added to a volume of 0.8 cc. 0.02 cc. (a sub-hemolytic dose) of fresh chicken serum (complement) was then added. The tubes were incubated for one hour. Rabbit red cells were then added and the incubation was continued for another hour. Hemolysis appeared as recorded under Set 1, Table I. A control set was run in which the cells, heated serum and saline were mixed and incubated for one hour. Two hundredths cc. of fresh chicken serum (complement) was then added and the tubes reincubated for one hour. No hemolysis occurred, as shown in Set 2. The hemolytic titer of the fresh serum which was used as complement was 0.1 cc. (Set 3, Table I). Five hundredths cc. caused only a trace of hemolysis. The amount used as complement (0.02 cc.) was of itself entirely non-

TABLE I.

Reactivation of chicken serum with chicken serum.

Tubes . . .	1	2	3	4	5	6	7	8	9	10	11	12	13
Serum. . . .	(1–0 0.6	0.4	0.3	0.2	0.1	.(1–16) 0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.5
Set 1*. . . .	4	4	4	4	4	3	+	±	0	0	0	0	0
Set 2.	0	0	0	0	0	0	0	0	0	0	0	0	0
Set 3.	4	4	4	4	4	±	0	0	0	0	0	0	0
Set 4.	4	4	4	4	4	4	2	±	0	0	0	0	0

* Set 1 shows the titer of fresh chicken serum; Set 2, inactivated chicken serum and fresh chicken serum as complement, ordinary method; Set 3, inactivated chicken serum and fresh chicken serum as complement, new method; Set 4 is the same as Set 1 with the exception that each tube contains 0.02 cc. more of fresh chicken serum than is indicated.

hemolytic. To test further the intrinsic hemolytic power of the complement serum, 0.02 cc. (dose of complement) was added to each of a series of tubes in which the titer of the fresh serum was being determined. This addition only slightly raised the end titer (compare Set 4 with Set 1, Table I). These controls show that reactivation was actually effected.

The results given in Table I were obtained with serum inactivated at 56° C. fifty-three degrees C. is the lowest temperature at which the serum can be inactivated within 30 minutes. It was found that reactivation can be effected by the method just described after the serum has been inactivated at temperatures between 53° C. and 58° C. Serum heated at higher temperatures was not reactivated.

The reactivation of chicken serum with guinea pig serum, new method.—The technique followed here was the same as that used in the experiment just described in which the chicken serum was reactivated with sub-hemolytic doses of the fresh serum of the same species. The results are recorded in Table II. Five hundredths cc. of fresh guinea pig serum was used as the dose of complement. It took four times this quantity of the serum to hemolyze completely a unit of rabbit corpuscles, twice the amount giving only partial hemolysis, and in controls containing one unit of the complement serum no hemolysis occurred. Here, as with the fresh chicken serum as complement, no hemolysis occurred when the three ingredients—cells, heated serum and complement—were put in the tubes at the same time or when the cells and heated serum were incubated before the complement was added. Reactivation was effected, however, when the heated serum and complement were mixed and incubated one hour before the cells were added. In this instance the chicken serum could not be reactivated if heated above 55° C. It has already been pointed out that 53° C. for 30 minutes completely inactivates the serum (see Table II).

TABLE II.

Reactivation of chicken serum with guinea pig serum.

Tubes....	1	2	3	4	5	6	7	8	9	10	11	12	13
Serum....	(1–0) 0.6	0.4	0.3	0.2	0.1	(1–16) 0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.05
53° C.....	+	2+	4+	3+	±	0	0	0	0	0	0	0	0
54° C.....	4+	4+	4+	3+	0	0	0	0	0	0	0	0	0
55° C.....	4+	2+	+	0	0	0	0	0	0	0	0	0	0

SUMMARY.

It has been shown that the natural hemolysins of chicken serum for rabbit corpuscles can be reactivated both with fresh chicken serum and guinea pig serum after heat inactivation. The reactivation is effected by mixing and incubating the inactive serum and complement before the corpuscles are added.

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THE THERMAL-DEATH POINT OF THE HUMAN INTESTINAL PROTOZOAN CYSTS.*

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In an experimental study to determine the thermal-death point and the general resistance of any organisms, or their cysts (spores), a criterion is always necessary to indicate whether the organisms are alive or dead.

The growth of bacteria upon artificial media has been employed as a criterion of viability in studying the resistance and the thermal-death point of this particular group of the Protista. But in a study of the cysts of the protozoa, parasitic in the intestine of man, this criterion is inapplicable, since it has been, thus far, impossible to cultivate the intestinal protozoa, with the exception of *Endamoeba histolytica* and *Endamoeba coli*, from their cysts. Yoshida (1920) has been able to cultivate *E. coli* and *E. histolytica* by causing excystation in an artificial medium composed of agar and defibrinated horse blood, but the method was attended with unsatisfactory results as a whole, for only 3 of 11 experiments made on *E. tetragena* (*histolytica*) and only 7 of 19 on *E. coli* were successful. It was therefore inadvisable to use growth upon artificial media as a criterion of viability, since it was only indifferently successful in its application to the study of but two of the parasitic protozoa.

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Another and valid criterion of viability which was used in the study of bacteria depended upon the ability of bacteria to produce an infection when inoculated into laboratory animals. This criterion could be employed to determine the viability of the cysts of *E. histolytica* and *Giardia intestinalis,* since infections in certain animals have been obtained with these organisms. It is possible to feed the cysts of *E. histolytica* to a kitten and obtain an infection, but not all attempts to produce an infection are successful. To obtain the desired results the cysts should be procured from a recent stool, and at the same time there should be a large supply of them, for with old cysts, and with a small number, the chances of obtaining an infection are very small. These conditions could not be met in this study, as only a limited supply of the cysts of this parasite were available.

Fantham and Porter (1916) produced an infection in mice and kittens by feeding them the cysts of *Giardia intestinalis.* But there is a grave danger in using these animals in testing the viability of the cysts of *Giardia intestinalis,* since they are often found parasitized by a species of Giardia, which does not differ materially from that living in man. Such a circumstance would lead to confusion unless proper precautions were taken, and these were too time-consuming to be practicable. But even if it were practicable to use the criterion of viability based upon animal infections, and satisfactorily determine the viability of cysts of *E. histolytica* and *Giardia intestinalis,* it would have had to be discarded on account of the fact that no infections have ever been obtained with *E. coli, Endolimax nana, Iodamoeba bütschlii,* and *Chilomastix mesnili,* the other species of cysts used in this study.

It was clear that neither of the above criteria of viability were practicable in their application to the study of all the different cysts, and this made it necessary to search for a criterion which could be uniformly utilized for all the cysts in the determination of their resistance and thermal-death point.

Kuenen and Swellengrebel (1913) were the first persons to use a dilute aqueous solution of eosin as a criterion of viability in the study of the cysts of *E. histolytica.* This stain is usually employed in the concentration of 1 : 1000. The above investigators held that this staining solution colored the dead cysts upon its penetration through the cyst wall, while the living cysts were not affected by the stain and remained very refractive in appearance. They also applied the test to the vegetative forms of *E. histolytica.*

Kuenen and Swellengrebel believed that the use of the eosin solution was a severe test in ascertaining the death of the cysts. They held to the rule that all stained cysts were dead, and that all the cysts which did not take the stain were viable. These investigators noted that the cytoplasm of the dead cysts became coarsely granular in appearance, and that it shrank away from the cyst wall for a short distance. When motile amoebae were killed and stained by the eosin solution, a dissolution of the cytoplasm took place, leaving the nucleus as the only remnant of the original organism.

After Wenyon and O'Connor (1917) had placed cysts of *E. histolytica* in boiling water and in strong disinfecting agents, and found that they stained pink in the eosin solution, while other cysts not similarly treated remained clear and refractive, they concluded ". . . that the eosin-stained cysts are dead, though it may be argued that others which do not stain may be dead also or, at any rate, non-infective."

Cutler (1920) added experimental proof in support of the eosin criterion of viability in his study upon the cysts of the soil protozoa. This investigator was able to bring about excystation of the cysts of the soil protozoa which were not stained by the eosin solution, but he was unable to do so with the cysts which had taken up the stain in their cytoplasm. Cutler's experiments were not sufficiently guarded from error, however, as to preclude the probability that a number of the unstained cysts, also, did not excyst, for the number of cysts at the beginning of the experiment was only estimated, and likewise the number of excysted amoebae. It is probable, then, that not all unstained cysts underwent excystation.

Yoshida (1920) was another investigator who employed the eosin-criterion of viability in his experiments on the reproduction in vitro of *Endamoeba tetragena (histolytica)* and *Endamoeba coli* from their cysts. He noted that sometimes the cysts did not take the stain, " although their nuclear and plasmic structures in stained preparations were shown to have degenerated or to have disappeared. . . . The living cyst is pale and markedly light-refracting, its cytoplasm is generally irregular, in large granules, and it appears rigid. The dead cysts which refract light slightly can be stained with eosin, while those which are markedly light-refracting do not take the stain.'' He concluded, as a result of his experiments, ''. . . that cysts taking the eosin stain are dead, but not, on the other hand, that all dead cysts absorb this stain.'' This conclusion was based upon experimental data and substantiated the theoretical conclusion of Wenyon and O'Connor given above.

It seemed advisable, however, to use the eosin-criterion of viability in determining the thermal-death point of cysts of parasitic protozoa of man, since it could be applied with uniformity to the study of all the different species of cysts. Although it was shown by other workers, and also noticed in my own experiments, that some of the cysts seemed dead from the appearance of their plasmic alterations, but did not stain, such cysts were held as viable, for no exceptions were taken to the fast lines of distinction between things alive (unstained) and things dead (stained) which the eosin-criterion of viability imposes. The eosin-criterion may err—*i.e.*, not all dead cysts may take up the stain yet it is a safe criterion to adopt, for, as Wenyon and O'Connor stated, ''. . . if we accept the eosin test as a criterion and regard all unstained cysts as living, the error in judgment will be on the safe side.''

The observations and experiments upon all the species of cysts were made at Baltimore, excepting those on the cysts of *Iodamoeba bütschlii* and *Endolimax nana;* these were made at the Hygienic Laboratory of the United States Public Health Service, at Washington, D. C. I am indebted to Miss Katherine Flower for the preparation of some of the experiments upon these particular cysts. I also wish to thank Dr. Charles E. Simon for some of the *Giardia intestinalis* material used in this study.

THERMAL-DEATH POINT.

Technique.—From the work of Kuenen and Swellengrebel (1913), and also from the observations made upon the experiments by the author, it was apparent that the stools should be washed several times in order to obtain the cysts free from the great numbers of bacteria and the presence of the chemical products of decomposition in the feces, which exert a detrimental influence upon the vitality of the cysts. In one experiment with the cysts of *Endamoeba histolytica,* Kuenen and Swellengrebel (1913) showed that the cysts in one batch, which were kept in a glass of water in which there was a marked growth of bacteria, lived only 16 days, while the cysts in another batch, which were kept in another glass of water containing a sparse growth of bacteria, lived 29 days.

In some preliminary experiments by the author to determine the thermal-death point of the cysts of *E. histolytica,* the feces were not washed to secure the cysts free from the large numbers of bacteria and the putrefactive products, but simply broken up in a bottle of distilled water. In this bottle a pellicle soon appeared upon the surface of the water, which showed that the bacteria were not inhibited in their growth. Eighty per cent. of the cysts were dead (stained with eosin) 4 days after they had been immersed in the distilled water, and this was only 5 days after they had been passed in the stool. The thermal-death point was found to be at 54° C. in 5 different series of readings; this point, as it will be seen later, was 12° to 14° C. lower than that reached with cysts taken from the same fecal specimen, but which had been separated from the bacteria and the putrefactive substances present in the stool.

Similar results were obtained in experiments made with the cysts of *Iodamoeba bütschlii.* In one experiment the stool was not washed, but only a very small lump of feces containing the cysts was placed in each of the several test tubes along with a little distilled water and then subjected to different temperatures. The thermal-death point occurred at 54° C.; this was 12° C. lower than the thermal-death point obtained with cysts which had been washed free from the bacteria and chemical substances of putrefaction of the stool. In 2 other experiments the cysts were not washed free from the bacteria and putrefactive products in the feces, but were simply suspended with some of the feces in a bottle of distilled water, then comminuted and allowed to stand. The medium had a profuse bacterial growth and a pungent odor. Cysts taken from this supply also showed a lowered resistance, and the thermal-death point was at 56° C. in one series, and 54° C. in the other.

These experiments demonstrated that the presence of the bacteria and the chemical products of putrefaction of the stool affected the vitality of the cysts by lowering their resistance, which resulted in an abnormally low thermal-death point.

Accordingly in all subsequent attempts to secure the different cysts for the experiments on thermal-death point determinations the stool was comminuted in distilled water, strained through one or two layers of cheese cloth to catch the large particles in the feces, and then allowed to sediment out in a bottle. The cysts fell to the bottom of the bottle along with the finer debris. The supernatant fluid was drawn off and new distilled water added. The sediment was then shaken up into a suspension again and allowed to settle a second time and the supernatant fluid again withdrawn. The process was repeated until the supernatant fluid was clear, which in the early washings was colored and contained large numbers of bacteria and the soluble putrefactive substances. The cysts were transferred with the sediment to another bottle which contained about 50 to 100 c.c. of distilled water, and kept in a cool place where the temperature varied from 12° to 22° C. This low temperature inhibited the excessive proliferation of the bacteria in the bottle, for at no time was a pellicle seen upon the surface of the water, nor was any odor ever detected emanating from the bottle to indicate rapid putrefaction. In these supply bottles the cysts were found in the fine superficial layer of silt which had precipitated after the coarser debris had first settled to the bottom.

To determine their resistance to various temperatures and the thermal-death point, the cysts were withdrawn with a little sediment and water by means of a pipette and then placed in small test tubes of the size employed in the Wassermann test. The sediment and water containing the cysts in each tube comprised about 0.5 c.c. in volume. The cysts in the test tube were placed in a water bath after a given temperature had been reached and kept there for 5 minutes. Precautions were always taken to maintain the proper temperature for the entire 5 minutes.

In the experiments to determine the thermal-death point of any of the different cysts, the cysts were subjected to temperatures having an interval of 2° C. between them. This small interval made it possible to study the resistance of the cysts to gradual increases in temperature and also the determination of the number of dead (stained) cysts at these temperatures. After their exposure, some of the cysts were taken from the test tubes and examined upon the same day or allowed to wait until the following day, in order to ascertain how many were dead. No marked difference was noticeable in the results of the examinations made upon the cysts the same day that they had been exposed to the temperature, and the results of the examinations of the cysts made on the following day. The thermal-death point was the same in most cases.

Examinations.—The examination of the cysts was as follows: A drop of the sediment containing the cysts was taken from each of the test tubes by means of a pipette and deposited upon a glass slide. There it was rubbed up in a small drop of the eosin solution (a 1 per cent. concentration was used in these investigations). A coverslip was then placed upon this stained wet-preparation. In the examination of the stained wet-preparation, a "reading" constituted the counting of the first 50 cysts observed, and recording the number out of these which stained (dead). Therefore the figure recorded in the tables to follow under each interval of temperature always represents the number of stained cysts found in the first 50 cysts encountered. These stained cysts had died as a result of their exposure to the different temperatures. A control reading was made for each series. This reading was made upon cysts which had not been subjected to any heat previous to their examination. They were taken from the same supply bottle as were the cysts which had been exposed to the high temperatures in the determination of the thermal-death point.

A neutral red solution was also tried instead of the eosin solution as a test of viability, but it had no advantage over the latter. Root (1921) used the neutral red solution to determine the period of survival of the cysts in the gut of the fly, and found that 2 to 4 per cent. more cysts stained by the neutral red than by the eosin, and he concluded that the neutral red test was a little more delicate compared to the eosin criterion. A difference, however, of 2 to 4 per cent. means only 1 or 2 additional stained cysts, since only the number of living cysts were recorded out of the first 50 cysts encountered, and a variability of this slight extent very often occurred between 2 successive readings for any interval, when using either neutral red or the eosin solution. It seems likely, therefore, that the difference of 2 to 4 per cent. might well be included in the scope of experimental error, and that solutions of neutral red and eosin are equally efficient as indicators of death.

Definition.—Several series of readings were made for the cysts of the different parasitic protozoa, and in most cases the temperature at which all the cysts were found stained by the eosin, in the majority of the series, was considered the thermal-death point. As a rule it was found that the different series of readings of any one species of cysts did not vary materially from each other, and that the thermal-death point was generally constant within 2° C.

The thermal-death point was determined for the cysts of the following parasitic intestinal protozoa of man: *Endamoeba histolytica, Endamoeba coli, Endolimax nana, Iodamoeba bütschlii, Giardia intestinalis* and *Chilomastix mesnili.**

Endamoeba histolytica cysts.

The cysts of *E. histolytica* were obtained from two sources. The cysts used in the first 4 series of readings (Table 1) to determine the thermal-death point were passed in a stool on December 8, 1919, by a patient who was undergoing treatment for amoebic dysentery. The cysts which were used in the last 4 series of readings came from the feces of a healthy carrier on December 14, a year later. The cysts from both sources did not differ in proportion, both belonging to the same size race. When unstained they measured from 9 to 12 microns in diameter; those with a diameter of 10 to 11 microns predominated. The cysts from the first supply contained no chromatoidal bodies. A small minority of the cysts of the second supply showed these bodies to be feebly developed. In both supplies the cysts contained either 1, 2 or 4 nuclei; the 4-nucleated cysts were the most numerous. The cysts containing 1, 2 or 4 nuclei appeared equally susceptible to the high temperatures.

TABLE 1.

Series of readings to show the thermal-death point of the cysts of E. histolytica at 64° to 68° C.

No. of Series.	Date of Series.	Cysts passed.	Control.	48°	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	Date Examined.
1	Jan. 28, 1920	Dec. 8, 1919	1	12	11	12	18	22	38						Jan. 29, 1920
2	Jan. 29, 1920	Dec. 8, 1919	1						35	43	42	48	50		Jan. 29, 1920
3	Feb. 6, 1920	Dec. 8, 1919	3				28	40	47	46	48	50			Feb. 6, 1920
4	Feb. 10, 1920	Dec. 8, 1919	4					34	44	46	49	50			Feb. 10, 1920
5	Dec. 23, 1920	Dec. 14, 1920	5				19	30	32	41	42	45	48	50	Dec. 24, 1920
6	Dec. 23, 1920	Dec. 14, 1920	5				19	33	35	40	43	44	47	50	Dec. 24, 1920
7	Dec. 27, 1920	Dec. 14, 1920	8				25	29	38	41	45	47	49	50	Dec. 28, 1920
8	Dec. 27, 1920	Dec. 14, 1920	8				23	30	33	38	46	48	49	50	Dec. 28, 1920

The first 4 series of readings (Table 1) were made 6 to 8 weeks after the cysts had been passed with the feces. These cysts, therefore, had undergone some degeneration. The thermal-death point in these series occurred at either 64° or 66° C. A year later, beginning December 23, 1920, 4 more series were made with the cysts obtained from the feces of the healthy carrier. In these cysts little or no degeneration had taken place, for they were only somewhat over 1 week old when the first series was made. This is the 5th series in Table 1. The control showed only 5 dead cysts out of the first 50 counted. The 5th, 6th, 7th and 8th, all made from the second supply of cysts, showed the temperature of 68° C. to be the thermal-death point.

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* The nomenclature employed to designate the different human intestinal protozoa is that most current in use, and it was for this reason alone, that the above terms were thought advisable. Until an opinion is forthcoming from the International Commission on Zoological Nomenclature, it seems best to apply the names of more common usage to the parasites and not to complicate the problem further by giving other generic and specific terms.

The thermal-death point in the last 4 series was 2° C. higher than that found for the cysts used in the first 4 series of readings. The rise in the thermal-death point was probably due to the use of fresh cysts as compared to those used in the first 4 series. It was also accounted for by the fact that the last 4 series were made within a short time after the cysts were passed out of the human body, while the first 4 series occurred within 2 weeks of each other, but from one to two months after the passage of the cysts in the feces. Hence it would appear that in view of the fresh condition of the cysts of the last 4 series the thermal-death point of 68° C. was closer to the truth than that of 64° and 66° C. in the earlier readings. It is more than probable that the resistance of the cysts used in the first 4 series had been lowered by some outside factors, such as the presence of bacteria and putrefactive substances, which had collected in the supply bottle from which the cysts were taken for the experiments. Although the temperature (12° to 22° C.) was low and inhibited the rapid proliferation of the bacteria in the bottle, yet by the time the first 4 series of readings were made, which was 6 to 8 weeks after the cysts had been washed and placed in the bottle, it was probable that the bacteria had multiplied to such an extent that they and the products of putrefaction had accumulated to the detriment of the cysts. The lowered resistance in these cysts resulted in the lower thermal-death point.

An average series of readings (Table 2) was computed from the

TABLE 2.

An average series of the cysts of E. histolytica to show the increase in mortality with each increase in temperature.

	48°	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°
Cysts of *E. histolytica*...	12	11	12	22	31.1	37.7	42.1	45	47.4	48.6	50

8 series given in Table 1. At the lower temperatures, 48° to 52° C., which were 10° to 14° above that of the human body, the cysts were very resistant. At 52° C. only 12 cysts were stained out of the first 50 cysts observed in the examination, or 24 per cent. When the cysts were exposed to temperatures higher than 52° C., the number of dead cysts increased with each increase of temperature, until all of them were dead at the thermal-death point of 68° C.

Endamoeba coli cysts.

The supply of cysts of *Endamoeba coli* was obtained from the feces of a patient who resided in Mississippi; the specimen was received on January 15, 1920. The stool upon examination showed a great number of *E. coli* cysts which measured from 15 to 21 microns in diameter, and also a large number of motile *Amoeba limax*. On February 5, 1920, the stool was washed and the cysts were placed in a bottle with distilled water, in the same manner as in the preparation of the cysts of *E. histolytica*. At this time the motile forms of *Amoeba limax* had all encysted; the cysts were present in aggregates resembling clusters of grapes. These cysts were small, measuring about 9 microns in diameter, and possessed a single vesicular, visible and centrally located nucleus and a very rough and thick wall. On account of these characteristics they led to no confusion in the examination of the *E. coli* cysts. The cysts of *Amoeba limax* often remained alive after their subjection to a temperature of 76° C. for 5 minutes.

The first series of readings to determine the thermal-death point of the cysts of *E. coli* was made on February 10, 1920, when the cysts were more than 3 weeks old, and after their washing 5 days previously. The first series of readings (Table 3) ran from 52° to 70° C. and demonstrated that the thermal-death point lay beyond 70° C., since only 39 cysts were dead out of the first 50 observed. It also showed that the temperatures of 52° to 64° C. had very little effect upon the cysts, for the number dead remained about the same for the intervals between these limits. The cysts began to show marked susceptibility to only those temperatures above 64° C.

In most of the following series of readings the thermal-death point of the cysts of *E. coli* was at either 74° or 76° C. In the 6th and 7th series of February 25 and March 3, however, the thermal-death point fell to 72° C. As in the case of the cysts of *E. histolytica*, when a fall in the thermal-death point occurred, the decline was due in all probability to some outside influence, such as bacteria or chemical sub-

TABLE 3.

Series of readings to show the thermal-death point of the cysts of E. coli at 72° to 76° C.

No. of Series.	Date of Series.	Cysts passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°	74°	76°	Date Examined.
1	Feb. 10, 1920	Jan. 15, 1920	7	7	12	10	13	14	13	14	14	—	19	39				Feb. 10, 1920
2	Feb. 12, 1920	"	5									19	18	41	45	45	50	Feb. 12, 1920
3	Feb. 17, 1920	"	4									—	26	41	49	50		Feb. 18, 1920
4	Feb. 18, 1920	"	4									27	35	45	49	50		Feb. 19, 1920
5	Feb. 20, 1920	"	4									35	46	47	49	50		Feb. 21, 1920
6	Feb. 25, 1920	"	8									31	40	43	50			Feb. 25, 1920
7	Mar. 3, 1920	"	24									45	45	49	50			Mar. 4, 1920
8	Mar. 8, 1920	"	19									37	—	42	46	49	50	Mar. 9, 1920
9	Apr. 14, 1920	"	12									38	39	—	41	48	50	Apr. 15, 1920

stances, which lowered the resistance of the cysts and caused their early death. A part of the proof for this statement is based upon the experimental data given above, showing how the presence of large numbers of bacteria and the products of putrefaction resulted in a lower thermal-death point of the cysts of *E. histolytica* and *Iodamoeba bütschlii*, and also upon the fact that this outside influence which increased the rate of mortality and lowered the thermal-death point of the cysts of *E. coli* on February 25 and March 3 was only temporary, for the thermal-death point was again back at 76° C. on March 8. The resistance of the cysts was only lowered for the brief period from February 25 to March 8, after which time those cysts which did not succumb regained their original power of resistance.

More proof of the temporary presence of this influence may be seen by a study of the controls of the different series. In series 1, February 10 (Table 3), 7 cysts were dead in the control, and this number decreased to 5 in the 2d series on February 12, and again to 4 in the 3d series on February 17, at which number it remained until February 25. On February 25, in series 6, the number of dead cysts in the controls increased to 8, and later in series 7, on March 3, to 24; after which a decrease ensued, with 19 cysts dead on March 8, and only 12 in the last series on April 14, 1920. The period when the outside influence was present in the bottle which lowered the resistance of the cysts and their thermal-death point coincides exactly with the period when a large number of cysts were dead in the control. This fact was proof that the increased death rate among the cysts was due directly to this outside temporary influence.

The reason the controls of March 3, March 8, and April 14 did not show a larger number of dead cysts than they did, since the examination upon these dates was made after the period of an increased rate of mortality, was due to the disappearance of the stained cysts which had died some time previously, through plasmolysis and disintegration. On these dates many empty cysts were found in the course of the examination.

It was found that in a supply of cysts in which there was an increased death rate, which had been preceded and followed by a slower one, that the small number of dead cysts which occurred as a result of a slow death rate at once became much larger in the period characterized by an increase in the rate of mortality, and that after this latter period of a rapid death rate the number of cysts became less again as a direct result of two factors—the resumption of a slow rate of mortality and the disappearance of the dead cysts through plasmolysis and disintegration. Had the dead cysts, which died during the previous period of a rapid death rate, not disappeared, then the number of dead cysts would have been larger when the slow death rate was resumed again.

TABLE 4.

An average series of readings of the cysts of E. coli to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°	74°	76°
Cysts of *E. coli*....	7	12	10	13	14	13	14	14	33	33.5	43.3	47.3	48.6	50

In Table 4 are the figures of a series of readings for the cysts of *E. coli,* which represent an average of the 9 series in Table 3. This average series of readings shows the high resistance of the *E. coli* cysts at temperatures ranging from 52° to 64° C. At 64° C. 94 per cent. of the cysts of *E. histolytica* had succumbed to the heat, but Table 4 shows that only 28 per cent. of the *E. coli* cysts died after exposure to this temperature. The cysts of *E. coli* were less susceptible to the high temperatures than the cysts of *E. histolytica.* The thermal-death point at 76° C. is at least 6 degrees higher than that of *E. histolytica* cysts.

Iodamoeba bütschlii cysts.

The cysts of this parasitic amoeba were previously known as " I "-cysts, because they usually show one, and sometimes two, deep brown colored masses in the cytoplasm, in the presence of an iodin solution. The brown masses are composed of glycogen and each is situated in a vacuole. The glycogen disappears in the course of the technique used with a permanent stain, such as iron-hematoxylin, leaving the vacuole empty. In 1919 Dobell created the genus *Iodamoeba* for this parasite and the specific name *bütschlii* was retained from an earlier description by Prowazek in 1912, of what is perhaps the same parasite, and which was then called *Entamoeba bütschlii.* Dobell (1919) believed that Prowazek's *Entamoeba bütschlii* was identical with the motile organism of the " I "-cysts and now known as *Iodamoeba bütschlii* (Prowazek 1912 emend).

The cysts are very irregularly shaped and measure from 8 to 20 microns in diameter. The cysts most often encountered measured from 10 to 15 microns. They generally have only a single nucleus, but occasionally a 2-nucleated cyst may be found. The nucleus is invisible in an unstained preparation, but when the cysts are stained with a solution of iodin the nucleus becomes visible with a more or less refractive membrane and a large, brightly refractive and eccentrically situated karyosome. The latter appears to be lodged upon the membrane. In preparations stained with iron hematoxylin the large karyosome does not lie directly upon the nuclear membrane, and peripheral chromatin may be seen lying in the space between it and the nuclear membrane. The peripheral chromatin may often be seen to be agglomerated into a crescentic mass such as was first noticed by Brug (1919). The cytoplasm contains large refractive granules; these may be seen both in the unstained cysts and those stained with the solution of iodin.

Five series of readings were made to determine the thermal-death point of the cysts of *Iodamoeba bütschlii.* The cysts were all obtained from a single specimen of feces which was passed on December 15, 1920.

TABLE 5.

Series of readings to show the thermal-death point of the cysts of Iodamoeba bütschlii, at 62° to 64° C.

No. of Series.	Date of Series.	Cysts Passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	Date Examined.
1	Dec. 16, 1920	Dec. 15, 1920	0	4	8	9	21	45	48	49	50	Dec. 17, 1920
2	Dec. 19, 1920	"	0			16	42	47	49	50		Dec. 20, 1920
3	Dec. 19, 1920	"	0			17	44	45	49	50		Dec. 20, 1920
4	Dec. 21, 1920	"	0			11	24	43	46	49	50	Dec. 22, 1920
5	Dec. 21, 1920	"	0			12	22	38	46	48	50	Dec. 22, 1920

The 1st, 4th and 5th series of readings gave the thermal-death point at 64° C., while the 2d and 3d series showed that all the cysts had died at 62° C. (Table 5). The thermal-death point of 62° to 64° C. for the cysts of *Iodamoeba bütschlii* was lower than that of *E. histolytica* and *E. coli.* No doubt, therefore, the cysts are not so resistant as those of the other parasitic amoebae. But at the lower temperatures of 50° to 54° C. the cysts were strongly resistant to the heat. A great increase in the number of dead cysts occurred at 56° C. (Tables 5 and 6) over the number of dead at 54° C. Less than one half as many dead cysts were found at 54° C. than were found at

56° C. The number of dead cysts was gradually augmented at the subsequent temperatures below the thermal-death point.

In the examinations of the control of each series of readings no stained cysts were seen, but the cysts showed other changes. It was noticed that a gradual decrease in the amount of glycogen had taken place. On December 16 all the cysts had glycogen in their vacuole, but on December 22 only a small number of the cysts had the vacuole completely filled, while most of the cysts had either an empty vacuole or the vacuole contained less glycogen than originally, as it did not stain so deeply in the presence of the iodin solution.

TABLE 6.

An average series of readings of the cysts of Iodamoeba bütschlii to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°
Cysts of *Iodamoeba bütschlii*	4	8	13	30.6	43.6	47.6	49.2	50

An average series of readings is given in Table 6; it was derived from the 5 series of readings given in Table 5. The larger number of cysts died after their exposure to the temperatures above 56° C. The thermal-death point was at 64° C. The cysts of *Iodamoeba bütschlii* might be expected to remain viable for a long period of time in a bottle with distilled water, if kept at a temperature from 12° to 22° C., for they were very resistant to temperatures below 54° C.; after 5 minutes' exposure to the latter temperature, only 18 per cent. of the cysts were dead. The great increase in mortality occurred at 56° C., when more than twice as many cysts died from exposure than at 54° C.

Endolimax nana cysts.

The *Endolimax nana* cysts were first described by Wenyon and O'Connor (1917) under the name of *Entamoeba nana,* but Brug (1919) showed that this organism should be classified under a new genus, since it differed greatly from the members of the genus *Entamoeba;* he accordingly assigned it to the genus *Endolimax* and retained the specific name of *nana.* Lately Dobell (1919) has added considerably to our knowledge of this parasite of man by describing the motile organism.

The cysts of *Endolimax nana* are generally ovoid in shape and measure from 8 to 10 microns in length (Dobell, 1919). They do not show any internal structure in unstained preparations, except for the presence of a few refractive granules. In iodin solution the cytoplasm usually stains yellow, and not brown, as is generally the case with the other species of amoebic cysts. The nuclei are very small and indistinctly visible. Each nucleus contains a karyosome which may appear brownish when viewed in one focal plane, and refractive when seen in another. The karyosome is eccentrically situated in a clear area and upon the nuclear membrane. The nuclei are from 1 to 4 (sometimes 8) in number. There is no peripheral chromatin. The nuclear membrane is generally indistinct in specimens stained with iodin, but visible in those well decolorized after staining by iron-haematoxylin. The presence of the small granules which in iodin solution appear brownish and refractive, depending as in the case of the karyosome upon the particular focal plane in which they are viewed, may be confused with the nuclei at first sight, but these granules are not situated in a clear area as are the nuclei. Dobell (1920) claims that the cysts do not contain any vacuoles or chromatoid bodies. Cysts containing glycogen, however, were encountered on rare occasions, but it was not clear whether the glycogen was situated in a vacuole, as in the cysts of *Iodamoeba bütschlii,* or localized within the cytoplasm itself, as in *Giardia* cysts. The glycogen body, accordingly, was not so definitely set off in the cytoplasm as in the cysts of *Iodamoeba bütschlii.* Cysts have also been seen which contained small bodies of varied shapes which stained like chromatin. It is possible that they represented chromatoid bodies, but again they may have been inclusions of another nature.

The cysts used in the experiments to determine their thermal-death point were characterized by the description given above, except that the size was more variable. The cysts measured from 6 to 12 microns in their greatest diameter. They were both ovoid and spheroidal in shape. In all other respects it may be said that they answered to the description given by Dobell (1920, pl. 2, fig. 10). The stool containing the cysts of *Endolimax nana* was passed on February 4, 1921.

Eight series of readings (Table 7) were made to determine the thermal-death point of this species of cyst. With the exception of the first series of readings, the thermal-death point occurred at 64° C. A fair degree of uniformity existed in the number of dead cysts for each interval of temperature in all the series of readings. There were two striking features of the 8 series. The first was the remarkably

TABLE 7.

Series of readings to show the thermal-death point of the cysts of Endolimax nana, at 64° C.

No. of Series.	Date of Series.	Cysts Passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	Date Examined.
1	Feb. 7, 1921	Feb. 4, 1921	0			10	13	20	35	50		Feb. 8, 1921
2	"	"	0			7	14	19	37	49	50	"
3	Feb. 8, 1921	"	0	1	6	6	9	18	36	47	50	Feb. 9, 1921
4	"	"	0	3	4	5	10	20	40	45	50	"
5	Feb. 9, 1921	"	3			7	13	19	36	49	50	Feb. 10, 1921
6	"	"	3			9	12	17	38	45	50	"
7	"	"	3			6	14	18	32	46	50	"
8	"	"	3			10	13	18	38	46	50	"

small number of dead cysts which occurred at 50° C., for only 4 per cent. of the cysts were found stained. These cysts proved to be the most resistant of all species at this particular interval. The other striking feature of the series of readings was the great increase in mortality which occurred at 60° C. over that of the preceding interval of temperature. In nearly all the series there were twice as many dead cysts at 60° C. than at 58° C. This is shown in Table 8, where an average of 18.6 cysts were dead at 58° C., while an average of 36.5 cysts were dead at 60° C.

TABLE 8.

An average series of readings of the cysts of Endolimax nana to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°
Cysts of *Endolimax nana* ..	2	5	7.5	12.2	18.6	36.5	47.1	50

The average series of readings in Table 8 was made from the 8 series given in Table 7. This series showed how remarkably resistant the cysts of *Endolimax nana* were to the temperatures of 50° C., but how there was a gradual increase in the number of dead cysts with each increase of temperature up to 60° C. At this latter temperature, however, a very marked increase of 36 per cent. occurred in the number of dead cysts. An increase of 21 per cent. took place at the next interval of 62° C., which was the same as that determined for the cysts of *Iodamoeba bütschlii* (Table 6).

On account of the similarity in morphology, except for size, of their nuclei, the cysts of *Iodamoeba bütschlii* and *Endolimax nana* may appear to belong to a single genus. Aside, however, from the other differences, purely in morphology, which is the reason for consigning these cysts to two different genera and species, is the evidence obtained in the experiments to determine their respective thermal-death point.

If we compare the series of readings for the cysts of *Iodamoeba bütschlii* with those for *Endolimax nana,* there are at once certain points of similarity, as well as others which are different, for each species of cyst. It will be seen from Tables 6 and 8 that the thermal-death point of both the cysts of *Iodamoeba bütschlii* and *Endolimax nana* was at 64° C. In this respect these two species of cysts are similar. This fact might be construed as evidence in favor of these cysts belonging to the same genus and species were it not for the additional fact that the readings differed from each other for the temperatures below the thermal-death point. The cysts of *Endolimax nana* were more resistant than those of *Iodamoeba bütschlii* at a temperature of 50° C., and whereas the great increase in mortality occurred at 56° C. (Table 6) for the cysts of *Iodamoeba bütschlii,* a similar increase in mortality occurred at 60° C. (Table 8) for the cysts of *Endolimax nana.* It was apparent from this study that the resistance of these two species of cysts was not the same when subjected to temperatures between 50° C. and 64° C., and the presence of this fact along with the differences in morphology does not seem to warrant placing these cysts in the same genus and species at the present time. Later, if it is shown that these organisms are but different size races of the same genus and species, or are otherwise generically related, then the differences in their resistance to high temperatures must receive a new interpretation, possibly from a physiological viewpoint, than that given above which appears as evidence in favor of separating these forms both as to genus and species.

Giardia intestinalis cysts.

The cysts of *Giardia intestinalis* which were used in the experiments to determine their thermal-death point were obtained from the feces of three different persons infected with this parasite. The cysts were ovoid in shape and varied in length from 10 to 15 microns and from 6 to 9 microns in width. Some of the cysts contained 2 and others 4 nuclei.

TABLE 9.

Series of readings to show the thermal-death point of the cysts of Giardia intestinalis, at 60° to 64° C.

No. of Series.	Date of Series.	Cysts Passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	Date Examined.
1	Feb. 10, 1920	Feb. 7, 1920	20	25	37	41	48	48	50			Feb. 10, 1920
2	Feb. 20, 1920	Feb. 19, 1920	0			45	46	48	48	49	50	Feb. 20, 1920
3	Feb. 21, 1920	"	7	21	41	45	46	48	48	50		Feb. 21, 1920
4	Feb. 25, 1920	"	22		41	46	47	49	49	50		Feb. 25, 1920
5	Mar. 1, 1920	"	19		45	45	46	46	48	49	50	Mar. 1, 1920
6	Mar. 3, 1920	"	23		43	46	47	47	49	50		Mar. 3, 1920
7	Mar. 15, 1920	Mar. 13, 1920	30		44	46	47	46	49	50		Mar. 16, 1920
8	Mar. 22, 1920	"	34		45	47	47	48	50			Mar. 22, 1920

Only 1 series of readings was made with the cysts taken from the first supply of cysts of February 7, 1920 (Table 9). In this series the control showed 20 cysts dead out of the first 50 cysts counted and the thermal-death point was at 60° C. The next supply of cysts of February 19 was a healthier lot, for the control showed that all the cysts were unstained. The second series in Table 5, which is the first series made from this supply of cysts, gave the thermal-death point at 64° C. This was 4° higher than that of the first series. The next four series (Table 9) were also made with the cysts from the second supply. The last two series of readings in Table 9 were made with the cysts taken from the third supply of March 30, 1920. Sixty per cent. of these cysts were dead when the first series of readings was made on March 15. But the resistance of the remaining viable cysts had not been altered, for the thermal-death point of this series was at 62° C., while in the next series it was 2° lower.

TABLE 10.

An average series of readings of the cysts of Giardia intestinalis to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°
Cysts of *G. intestinalis*.....	23	42.2	45.1	46.7	47.5	48.9	49.6	50

While the thermal-death point varied from 60° to 64° C., and although in the majority of the series it was at 62° C., it is more than probable that, providing the cysts are all viable to begin with, as in the second series of February 19, that the thermal-death point is 64° C. This thermal-death point is the same as that of *Iodamoeba bütschlii* and *Endolimax nana* (Tables 6 and 8), but lower than that of *E. histolytica* and *E. coli.*

As was done with the series of readings of the other species of cysts above, an average series of readings was also made for the Giardia cysts from the 8 series found in Table 9. The Giardia cysts were not very resistant to the lower temperatures, for at 52° C. 84.4 per cent of the cysts were dead. Two degrees lower, viz., at 50° C., they were much more resistant, since only 46 per cent. of the cysts stained with the eosin. The Giardia cysts were the least resistant to the high temperatures of all the different species.

Chilomastix mesnili-cysts.

The cysts of *Chilomastix mesnili* are the smallest of all the cysts of the different intestinal protozoa of man. They are lemon shaped and measure from 4.5 to 6 microns in width and from 7.6 to 9 microns in length. In an unstained wet-preparation they appear refractive and show no internal structure, save the presence of 3 or 4 very bright spots and a dull area to one side of the cysts, which, with the aid of the oil immersion, may be identified as the nucleus. The cyst wall is uniform in thickness except at the somewhat pointed end of the cyst, where it is slightly thickened.

The cysts of *Chilomastix* which were used in the tests to determine the thermal-death point came from the feces of a patient who has

TABLE 11.

Series of readings to show the thermal-death point of the cysts of Chilomastix mesnili, at 68° to 72° C.

No. of Series.	Date of Series.	Cysts Passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°	Date Examined.
1	Jan. 6, 1920	Jan. 2, 1920	0	5	4	9	8	12	13	15	29	47	50			Jan. 6, 1920
2	Jan. 15, 1920	"	1						11	15	28	44	50			Jan. 16, 1920
3	Jan. 28, 1920	Jan. 27, 1920	0						11	43	48	49	50			Jan. 29, 1920
4	Feb. 6, 1920	"	0						5	8	26	48	50			Feb. 6, 1920
5	Feb. 10, 1920	"	1						2	6	27	40	43	50		Feb. 10, 1920
6	Feb. 17, 1920	"	0						6	8	15	—	41	50		Feb. 18, 1920
7	Feb. 19, 1920	"	2						6	9	10	39	47	50		Feb. 19, 1920
8	Mar. 1, 1920	"	1						17	22	24	22	39	47	50	Mar. 2, 1920
9	Mar. 8, 1920	"	0						15	29	36	40	40	49	50	Mar. 9, 1920
10	Apr. 14, 1920	"	2								36	38	48	49	50	Apr. 14, 1920

carried the infection for more than two years. Only 2 batches of cysts were employed in securing the 10 series of readings given in Table 11.

The first and second series of readings of January 6 and 15, respectively, were made with the cysts of the first supply. The thermal-death point was 68° C. in both of these series. A remarkable resistance was shown to the temperatures from 50° to 60° C. when compared to that of the cysts of the other parasites.

The last 8 series of readings in Table 11 were made with the cysts from the second supply of January 27, 1920. The 3d series of readings in Table 11, or the first series made from the second supply of cysts, showed more cysts dead from exposure to the temperatures 62° to 64° C. than that which occurred at the same temperatures in any of the preceding or subsequent series. The cause of this increase in the death rate could not be learned or explained. The thermal-death point was at 68° C., as in the first two series of readings.

The next three series of readings of February 10, 17, and 19 showed a smaller number of cysts dead at 60°, 62°, 64° and 68° C. than in the earlier series, and the thermal-death point advanced 2 degrees to 70° C. Less than half a month later, on March 1, the thermal-death point advanced a second time; in this and the next 2 series of readings it was at 72° C.

It was impossible to find a satisfactory explanation for the advances of the thermal-death point of the cysts of *Chilomastix mesnili.* It is probable that the true thermal-death point is between 70° and 72° C., and that some factor which was present for a time in the supply bottle temporarily affected the resistance of the cysts, and at that time lowered the thermal-death point. With the disappearance of the influence, the cysts were gradually able to regain their normal resistance, establishing the thermal-death point at 72° C. in the last 3 series of readings.

This outside factor or influence was probably not bacterial and chemical in nature, as occurred in the preliminary experiments with the cysts of *E. histolytica* and *Iodamoeba bütschlii* which resulted in a decrease in the thermal-death point, as related above, for there were no indications of profuse bacterial growth in the supply bottle, nor was there any odor or pellicle present. The supernatant water was very clear.

The control column of readings in Table 11 presented striking results, for not more than 2 stained cysts were ever encountered.

This condition would lead one to believe that most of the cysts remained viable, and that the rate of mortality was practically nil. The cysts, however, were becoming scarcer, as was noticed from the length of time it required to find the 50 cysts in each preparation in each succeeding series. They were very few in number when the last series was made on April 14. This fact proved that the cysts were gradually dying in the supply bottle, from which the cysts were taken for the control readings. It was also apparent that the dead cysts were disappearing through disintegration in order to account for the small number of stained cysts encountered during the examination of the controls. Only a few cysts were observed which showed intermediate stages of plasmolysis in the control examinations and likewise a few empty cysts. The empty cysts, however, were so difficult of detection that undoubtedly they were often missed in the examinations. In view of this fact, the control readings can not be strictly correct. The death rate of the cysts in the supply bottle must have been very slow, since the temperature in the supply bottle was much lower than the temperatures of 50° to 62° C. in the experiments to determine the thermal-death point, and at which the cysts were so remarkably resistant.

TABLE 12.

An average series of readings of the cysts of Chilomastix mesnili to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°
Cysts of *Chilomastix mesnili*......	5	4	9	8	12	9.5	17.2	27.9	40.7	45.8	49.1	50

Table 12 gives an average series of readings which was computed from the 10 series given in Table 11. The series shows very clearly the evidence for the strong resistance of the Chilomastix cysts to the temperatures of 50° to 62° C., for at the latter temperature only 34.4 per cent. of the cysts were dead.

The number of dead cysts given at 60° C. is low, for 5 of the 9 readings given at this temperature in Table 9 showed 11 of 17 cysts were dead. The average reading at this temperature was lowered by the small number of dead cysts recorded in the readings of the 4th, 5th, 6th and 7th series. The thermal-death point in Table 10 is 72° C.

SUMMARY.

In the above experiments, with the aid of the eosin-criterion of viability, the thermal-death point of the different cysts of the parasitic intestinal protozoa of man was determined as follows: *Entamoeba histolytica,* 68° C.; *Entamoeba coli,* 76° C.; *Iodamoeba bütschlii,* 64° C.; *Endolimax nana,* 64° C.; *Giardia intestinalis,* 64° C., and *Chilomastix mesnili,* 72° C. In quoting the above temperatures as the thermal-death points, it will be remembered that they are subject to a slight downward deviation of generally 2° C., since all the experiments upon each species of cysts did not end in a common thermal-death point. The remarkable resistance of all the protozoan cysts to the high temperature is quite analogous to that of the spores of bacteria; in both cases the thermal-death point is much higher than that of their respective vegetative and non-spore bearing stage.

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ALASTRIM IN JAMAICA.*

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Plates XII to XX. (Figures 1 to 15.)

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In September, 1920, one of us was invited by Dr. Deeks, of the United Fruit Company, to go to Jamaica to observe the epidemic of Alastrim which had prevailed there since May, and the following description of the disease is based upon the impressions gained during a stay of twelve days in Kingston, together with the observations of Dr. Moody, who had been studying the clinical phenomena since the beginning of the epidemic. The United Fruit Company gave me their most generous assistance, and to their officers, and especially to Dr. J. L. McLean, my thanks are due.

The name Alastrim is of Brazilian origin and is derived from a Portuguese word meaning to spread or strew about, hence something spread, a mantle or cloak. Other names such as Amaas, Sanaga pox, Kaffir pox, or milk pox, and, indeed, many others, have been applied in various countries to what seems to be the same disease.

In all these epidemics, which have been numerous, the great question has concerned the identity of the affection with smallpox, and in spite of the continued attention which has been devoted to it, this question is still unsettled. It is not even known whether it has always been the same disease in the various epidemics, but at least they have had in common the main feature which seems to distinguish them from typical smallpox, namely, the mildness of the affection and the very low mortality. Cleland and Ferguson, Armstrong, Green and others describe an epidemic of this kind which occurred in Australia in 1914, in which the mortality was very low and in which

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Green found experimentally that vaccination with standard vaccine was less effective than in true smallpox. Dickson and Lasselle and Scheult described an epidemic in Trinidad, and Korte, Plehn and Grant have discussed the similar or identical Sanaga pox and Amaas in Africa. It is probably in Brazil that this condition has been most widespread, and there are papers describing it by Carini, Ribas and Aragao. The last named in his paper of 1911 states that in the epidemic then progressing there were at least 250,000 cases, with a mortality of only 0.5–2 per cent.

The descriptions given by these authors correspond minutely with what we have observed in Jamaica, and we have little hesitation in concluding that we were dealing with the same disease. It is less easy to be sure as to the relations between Alastrim and the mild forms of smallpox which have been observed in the southern United States in epidemic form from 1900–1905. The long-continued epidemic occurrence of a very mild form of smallpox in various parts of Canada suggests the possibility that there, too, the disease may be more closely allied to Alastrim than to the severe smallpox of other epidemics. Indeed, a survey of the older literature on smallpox shows that there have been many epidemics of exceedingly mild character and the whole difficult question of the general uniformity of smallpox is brought up.

In Jamaica the epidemic of 1920 appears to have started in Kingston in May, and it is thought that the infection must have been brought from Cuba, where there were many cases of this disease. Cuba is only a few hours distant, and there are frequent boats between Santiago and Kingston. But it may have come from South America, where it is reported that there were many cases. Traffic between the ports of the United States of Colombia and Kingston is direct, and less direct communication with other ports on the northern coast not very difficult.

The exact mode of introduction was not determined. From Kingston it spread throughout the island and a few cases were reported from each of many rural districts and villages. In September the number of cases in Kingston, at any one time, was variously estimated at 400–700, of which about 325 were isolated in a building outside the city.

The disease was recognized as Alastrim by Dr. Hunt, the S. M. O. in Kingston. The panic among the people which would have ensued upon the outbreak of an epidemic of severe smallpox was thus averted, and the Central Board of Health was able to do much to control the spread of the disease by systematic vaccination and revaccination. More than 100,000 successful vaccinations have been performed up to the present date. The government also gave all the help it could to the government bacteriologist in the work reported in this paper.

Course of the disease.

Through the kindness of Dr. Murray, the District Health Officer, we were able to follow the search for incipient cases, and thus see in their houses those in whom only the first symptoms of illness were appearing. All other stages could be observed at Bumper Hall, the isolation hospital.

In all, up to the present time, 2,333 cases have been reported. The illness begins after an incubation period which is generally thought to be fourteen days, but which in the course of an epidemic it is difficult to determine accurately. The following evidence bears on this point:

Case 1. Nurse at Bumper Hall, not vaccinated. Headache and pain in the back began fourteen days after she took up duty and was exposed to infection.

Case 2. Was up in Kingston for three days, the epidemic being prevalent in Kingston only, and returned to his country district, where no cases had hitherto occurred. Symptoms developed ten days after returning.

Cases 3, 4 and 5. Infants born at Bumper Hall, free of any signs of the disease. Nursed by their mothers. Rash appeared on the tenth, eleventh and twelfth days after birth.

Case 6. Exposed to infection, so far as he knows, only twice, once thirteen days before, and a second time three days before the onset of symptoms.

Case 7. Developed symptoms eleven days after leaving Kingston where he had been at a school in which some of the scholars were infected.

The onset is sudden with headache and fever, often with some general aching, malaise and vomiting. Apparently none of these symptoms are very severe in most cases, and a complaint of backache was generally elicited only by direct questioning, and then usually in the case of women. In children convulsions sometimes occur at the onset. In 202 cases the incidence of these various symptoms of onset was as follows:

Headache in 172 cases	85 per cent.
Pain in back in 111 cases	54 per cent.
Pain in limbs in 41 cases	20 per cent.
Vomiting in 32 cases	10 per cent.

Backache was severe in only forty-five cases; the other patients described their pain as moderate. The combination of headache, vomiting and pain in the back occurred in only twenty-one of the patients, and of these only six vomited more than once.

The temperature at the onset may rise to 104° or 105° F., but is generally in the region of 103° F. It persists with slight variations for three or four days and then rapidly falls to normal as the rash appears, or even before its appearance. The temperature then remains low for four or five days, but rises again as the rash matures. In mild cases there is no secondary rise of temperature, and in severe cases the rise appears to be dependent on the extent of the rash and the degree of bacterial infection of the skin, since there is only a slight rise when the pus is practically free from bacteria. The patient is often not conscious of the secondary fever, although his temperature may be 102° F. After a few days the temperature again returns to normal and stays there unless complications, such as boils, occur.

In a few of the very severe cases the fever may resemble the typhoid type, persisting for fourteen or fifteen days before falling to normal. The temperature at the onset is no sure index of the severity of the case.

Most of those seen in the pre-eruptive stage were not particularly sick, although they generally stopped work. The eruption appears after three or four days (21 times after 2 days, 75 times after 3 days, 70 times after 4 days, and 36 times after 5 days), and is usually first seen on the forehead, wrists and forearms. Of 202 cases, the location of the first rash as noticed by the patient was as follows: Face, 120; wrists and forearms, 52; both arms and face, 27; scrotum, 1; inner side of knee, 1; elbow, 1. The first sign of the eruption is usually in the form of a few small widely separated papules or nodules which are very superficially placed in the skin, slightly elevated, but not hard or shotty on palpation. During the next day or two many more nodules appear, and then suddenly a great number, so that the whole face and trunk, as well as the arms and legs, are studded with them. (Figs. 1 and 2.)

Other patients begin with the appearance upon the forehead and cheeks of a translucent reddish nodular or irregular thickening of the skin. The skin of the forehead becomes rigid and finely mammillated, like shagreen leather, and later when these nodules become pustular there is a great tendency to confluence. Elsewhere on the body the pustules are usually quite discrete and reach a much larger size. (See Figs. 3, 4, 5 and 6.)

Two forms could thus be distinguished, but in neither form could one observe any definite umbilication in the early stages.

The development of the rash is often accompanied by oedema of the subcutaneous tissues, especially about the nose and eyes. In the majority of cases this oedema is slight, but in others it is so bulky as sometimes completely to close the eyes. It appears on the third or fourth day of the rash, and reaching its height on the seventh or eighth day, rapidly disappears.

During the third and fourth days of the eruption the nodules assume a more vesicular appearance, and a clear or opalescent fluid can be withdrawn with a fine pipette—in each case only a very minute droplet. During the following days, the fifth to the ninth or tenth, the eruption becomes definitely pustular. The skin of negroes offers a poor opportunity for the observation of the details of the appearance of such eruptions, but in the white patients there could be seen a red halo about each pustule. The thin epidermis over the pustule itself is stretched, so that the surface glistens and all the pustules bulge forward as smooth hemispherical projections. About the eighth day of the rash the pus begins to be inspissated, and the middle of the roof of the pustule sinks and dries a little, so as to become somewhat opaque. This produces a distinct secondary umbilication, such as is also seen in smallpox, but this is not the characteristic umbilication produced by the epithelial strands in the early stages during vesicle formation (Fig. 7).

Further desiccation produces crusts which loosen readily and are rubbed off, each leaving in the negro a small patch of depigmented pink skin fringed with partly desquamated epithelium and surrounded by a zone of deep brown pigmentation (Fig. 8). There is, as a rule, no deep pitting or scarring, and although the pigmented spots remain for months, they gradually fade and the skin is left practically smooth (Fig. 9). Slight pitting occurs in some cases, but it is by no means so deep and disfiguring as that following smallpox.

With the appearance of the eruption the patients feel a sense of relief, the temperature falls, and there is little complaint of headache or other symptoms; but with the development of the vesicles and pustules there is burning pain in the affected skin which often keeps them awake at night. They are much relieved if any one will puncture their pustules and squeeze out the contents. This we did in many cases, primarily to secure the contents, and next day always found the eruption dried up and the patient much more comfortable. There is no noticeable odor about these patients, in which respect they are in sharp contrast with those affected with smallpox.

The soles of the feet and palms of the hands become studded with deeply sunken pustules which are painful and greatly disable the patient, since they can not stand nor grasp anything without extreme suffering. During the height of this disability they lie in bed, and some of them complain of being very ill and are drowsy or show great mental depression. Their appetite generally continues good, however, even in spite of the intense sore throat from which a few suffer. Suddenly, next day they are again bright and cheerful, and after that convalescence proceeds without interruption. But in most cases there is very little of the feeling of severe illness and the greater number of the patients move about and try to amuse themselves.

The eruption is usually distributed pretty evenly over the whole body, being more closely set as a rule on the face and forearms, where it first appears. There were few, if any, cases of typical confluent eruption over extensive body surfaces, although a number of patients presented a completely confluent pustular exanthem over the face which later, in healing, left a rough crusted and suppurating surface. In many instances there were, when the eruption was mature, little groups of pustules, all of which could be evacuated by pricking one. Frequently some irritation or the presence of some lesion of the skin independent of the disease led to the outburst of a closely set crop of pustules in one or more places. One man, a policeman, who wore a uniform very tight under his arms, developed an almost confluent patch of pustules in the margins of the axilla. Others who had scratched or abraded the skin showed corresponding groups of pustules. The scalp is very generally affected even in mild cases, but the lesions are usually few in number. Pocks are frequently present on the hard and soft palate, less often on the uvula, pillars of the fauces, and mucous membrane of the mouth. In a few cases the frenum of the tongue was affected. Hoarseness and aphonia have been noted in a number of the severer cases and lesions found in the larynx. In several of the fatal cases the larynx and trachea have been studded with pocks in the mucous membranes. The external genitalia are also affected, especially the prepuce, which becomes much swollen and gives rise to pain and difficulty in micturition. Pocks are generally present on the edges of the eyelids, although the infraorbital space is often left free. None have been seen on the conjunctiva. The eruption seems to be slightly more profuse on the back than on the front of the trunk. It is not more sparsely distributed between the knee and ankle than elsewhere.

Apart from the inconvenience and pain caused by the eruption, there are few constitutional disturbances, and complications are uncommon. Delirium was never noted. Menstruation did not occur in women unless a period were due, and even then none complained of excessive loss of blood.

Blood counts were made in forty-eight cases with the following results: Before the onset and for the first 3–4 days of the rash the leucocyte count is normal or much diminished. In only 3 cases was it as high as 10,000, and in the great majority it ranged between 2,000 and 7,000. As the eruption matures the count rises to about 15,000, and in a few cases in which the pus of the pustules was much infected with bacteria the leucocyte count was 25,000. Differential counts so far as completed show about the normal ratio, with a few myelocytes. There is no secondary anaemia.

The urine was examined in fifty cases, but albuminuria was never found except where it was readily explained by some other cause, such as an urethral discharge. In three cases there was bronchitis with blood-stained sputum, lasting a few days.

Boils formed the most frequent complication, coming on about the fifteenth day after the onset of the rash and proving sometimes very troublesome. Impetigo also occurred in several cases as the rash was disappearing.

A mild conjunctivitis sometimes arose in those cases in which there were pocks on the eyelids, but never led to serious results.

Laryngitis was fairly common. The patients complained of extreme sore throat, and there was sometimes swelling of the cervical lymph glands. Usually this passed off after a few days, but in some of the cases which were studied at autopsy there was a profound destructive change in the tissues of the fauces, the pharynx and the glottis, involving also the tonsils.

The prognosis is very good. Of 2,333 cases reported to date ten have died. Of these seven were adults, five women and two men. Of the women four were six or seven months pregnant, all of whom displayed a marked tendency to haemorrhage. The other case had a confluent rash and died on the eleventh day. Of the men, one was fifty-six years old and died during convalescence. The other had extensive lesions in the mouth and throat and was dying when admitted to the hospital. The children died within the first month of life, contracting the disease shortly after birth. Two macerated foetuses were born a month after the mother had an attack of alastrim, with what appear to be pocks on their skin.

HISTOLOGICAL CHANGES.

In order to make a study of the histological changes in alastrim, pieces of skin were excised from several cases with pocks in various stages of development. Of these the most instructive are the very early stages, and after studying these it seems that it would have been desirable to excise similar pieces during the pre-eruptive stage. Naturally some of the sections from such pieces of skin might not represent the future site of a pock, but it seems that in some places, at least, recognizable microscopical alterations could be found in the corium before the slightest evidence of the exanthem was visible to the naked eye. The late stages are of relatively little interest microscopically. The materials from several autopsies, including those in new-born infants, were also preserved for study.

The earliest stage of the eruption in our possession is from the patient Hall, who began to feel ill on September 9, and in whom the rash first appeared on September 14. Skin was excised on September 16—that is, on the third day of the rash. The appearance of this eruption can be seen in Fig. 3, taken at the time of the excision; Figs. 10 and 11 show the microscopical changes.

It is to be observed that in the corium involved in the projecting papule there is an intense inflammatory reaction, although changes in the overlying epithelium are as yet only in their inception. This must be interpreted as evidence that the infective agent begins its activity in the corium, extending later into the epithelium. It is quite clear, since the eruption bursts out almost simultaneously over the whole body, that the organism concerned must be carried by the blood stream. In view of the infectivity of the fluid contents of the pocks, any other explanation of their origin than this seems untenable. In that case it is not surprising to find the earliest evidences of its influence in the terminal capillaries of the corium and of its papillae, and there seems to be little support for the suggestion that this somewhat hypothetical organism is essentially a parasite of the epithelium.

The blood vessels in the depths of the corium are as usual surrounded by a few wandering cells. These are very well preserved mononuclears with some highly amoeboid cells with basophilic granules. As the upper layer of the corium is approached in the area of the beginning pock, the small blood vessels with their attendant lymph vessels become surrounded by a dense mass of cells, which spreads off into the crevices of the adjacent tissues. Polymorphonuclear leucocytes are numerous, especially among the cells which are wandering toward the skin surface away from the vessels, but most of those about the blood vessels are distorted and misshapen mononuclear cells among which definite plasma cells with bluish protoplasm and unstained halo about the nucleus may be seen. Certain areas of the superficial layer of the corium are especially oedematous and thickly infiltrated with wandering cells. The papillae of these areas are widened by oedema and contain wandering leucocytes. Over these the epithelium is in some cases quite unchanged, or at most shows a beginning vacuolation of the cells. In those places which can be definitely recognized clinically as beginning pocks the epithelium is much more relaxed and separated into loose cells. At some points in such areas there may be distinct rounded spaces evidently filled with fluid which presses aside the epithelial cells. Such spaces are generally loaded with leucocytes as well. But in these areas the affection of the papillae and superficial layer of the corium appears to be more advanced than that of the epithelium.

Wherever there are areas of infiltration of the corium, the overlying epithelium begins to show the remarkable vacuolation especially well described by Unna as balloon-like. The vacuoles seem to us to be in the nucleus of each cell, for they are always surrounded by a thin film of nuclear substance or a band of nuclear substance extends across the vacuole connecting its opposite walls. Occasionally the nucleus is flattened and pushed to one side, but in most cases it appears to inclose the vacuole. The separation of such cells by accumulations of fluid and leucocytes is often accompanied at this stage by the necrosis of the overlying epithelial cells which stain solid pink.

We were unable to observe umbilication in the early stages in this disease and there is no occasion for the discussion of the theories as to its production. We see no such fan-like necrosis of the central epithelium in a pock as was described by Weigert in his explanation of the umbilication. Spaces merely arise in the epithelial layer and coalesce into the cavity of the developed pock. There are sometimes numerous bridges of epithelium left, stretching between the top and the floor of the pustule in alastrim just as in smallpox.

The later stages constitute a progression of the same process. The vesicle appears to arise within the epithelial layer and to become gradually more and more filled with leucocytes. Around the margins of the pustule the lower layer of epithelium which forms the floor is unbroken, but in the middle in nearly all cases it is destroyed, and the dense infiltration of leucocytes is continuous from the corium into the cavity of the pustule. The infiltrated corium forms the floor of the pustules and the papillae disappear from this surface (Fig. 12).

Another early case, that of Burton, was studied by excision of a piece of skin on the third day of the rash. The vesicle (Fig. 13) is more definitely formed than in the case just described and lies chiefly in the upper layers of the epithelium, so that sections passing through the margin show it bounded below by the flattened keratohyalin layer and superficially by a hyaline pink staining layer of keratinized and necrotic cells. But when the center of the vesicle is approached it is found that at least two papillae of the corium with the oedema and infiltration described above project as it were into the cavity of the vesicle and are no longer covered by any remnant of the Malpighian layer. The extensive infiltration about the vessels of the corium underlying this pock is as conspicuous as in the other cases.

No bacteria have been found in the sections of such pustules, although they occasionally appear in smears of the purulent contents. When examined with dark-field illumination, certain minute granular bodies with slight dancing motion could be seen in great numbers in the fluid taken from the pustules. These were found also in the filtrate from the same material after passage through a Berkefeld filter. They correspond fairly well with the bodies described by Prowazek, Paschen and others, but we hesitate to express any opinion as to their nature in the lack of any evidence.

In sections through the skin lesions in the earliest stages there are to be seen small bodies lying among the leucocytes which surround the blood vessels in the corium, which resemble in some degree those described by Councilman and many other writers on smallpox. They are also found in considerable numbers between the cells of the epithelium, but are rarely seen within leucocytes or epithelial cells. They resemble very small rounded cells 1 to 4 microns in diameter, with a deeply stained, rounded, solid nucleus and a delicate irregular cytoplasm which takes a faint pink stain. Some have no recognizable cytoplasm. The irregularity in size and form makes it impossible to feel sure that they represent anything more than isolated cell fragments, but they are quite numerous in the affected areas, absent elsewhere, and are by no means always in any suggestive juxtaposition with distorted cells. When they lie in an isolated position between epithelial cells it seems difficult to interpret them as cell fragments. In later stages of the development of the pock many of them can be seen engulfed by leucocytes.

The histological description of such bodies—even including the Guarnieri bodies, which these may perhaps represent—has led to no useful result, owing largely, it seems, to the extreme irregularities in form which constitute so good a disguise for any questionable organism. Until some significance can be given to these structures, therefore, by experimental studies, including their isolation and the demonstration of their living nature, we can only look upon them as interesting details in the morphology of the lesion.

Healing of the pustules occurs in the way so often described for smallpox, but it leaves behind so little pitting or scarring that we must regard the changes described in the corium as slight when compared with those of smallpox, although they seem profound enough in the sections.

There were three autopsies upon the bodies of women, two of whom were pregnant, and autopsies were also performed upon several macerated foetuses born after the mother's attack of alastrim, and in most instances showing marks of pocks upon their skin (Fig. 14).

Case 1. A pregnant woman in whom death occurred on the twelfth day of the rash. She was admitted bleeding from the nose, mouth and uterus. The mouth and throat were lined with a thick layer of necrotic material which involved the larynx. The trachea showed pock-like elevations throughout its entire length, and even the bronchi showed traces of the same eruption. The lungs contained numerous scattered haemorrhages. Similar petechial haemorrhages were found in the skin, heart muscle, kidneys, bladder and intestine. No lesions were seen in the foetus. Post mortem decomposition had proceeded so far that the microscopical sections are of little value. Sections through the pocks in the trachea show a thickening of the epithelium with a dense covering of bacteria, but this elevation does not closely resemble one of the cutaneous pocks.

Case 2. Also a pregnant woman at the seventh month. The skin was covered with well-formed pustules. The pharynx and larynx were lined with necrotic material of a dark greenish-black color with much loss of substance. The trachea showed pocks throughout its entire length, and the bronchi were similarly affected. There were some small areas of broncho-pneumonia in the right lung. Haemorrhages were few, the largest being a subserous haemorrhage over the uterus. The first two feet of the jejunum presented a remarkable condition, being dark and haemorrhagic with a great deal of oedema. In places the lumen seemed occluded by a gelatinous mass produced by the thickening of the wall.

The tissues from this case were well preserved, but sections of the organs showed no especial abnormality except in the case of the intestine, in which the section shows an extreme oedema with haemorrhage. The lumen is filled with a coagulated material, but the section is insufficient to explain the nature of this process. Section of the trachea shows patches of swollen and necrotic epithelium which apparently represent what appeared on gross examination as pocks.

Case 3. A woman who died on the eleventh day of the rash. The pocks were scabbing on the face, but on the body were confluent in places, frequently haemorrhagic, and abraded. The throat was clear and the trachea showed no eruption. A few areas of lobular consolidation were found in the lungs. The other organs showed no evident alterations.

Microscopically all the organs were studied, but no changes referable to the alastrim were observed except those in the skin. The bone marrow of the femur is entirely composed of fat, with scarcely any myeloid cells.

Sections from the tissues of the macerated infants showed only tissues so badly decomposed as to be worthless. Sections taken through the areas thought to be pocks in the skin show an elevation of the whole epidermis with haemorrhage, but not the characteristic lesion as seen in the adult skin.

It is evident that the virus passes readily through the placenta into the foetal circulation, for of twenty cases admitted after attacks of alastrim, there were eight abortions at about the sixth month, and in each case the macerated foetus was marked with scars of the disease. In two cases the abortion occurred eight weeks after the onset of the disease in the mother. Two children were also born alive with marks of alastrim. One of these was born at full term with nine scars of pocks on the face, twenty-two on the trunk, and a few on arms and legs, including some on the soles and palms. The other child born at the seventh month had pigmented scars on the arms and legs. The mother had the onset of her attack eight and a half weeks before. The remaining ten women went through normal labor and gave birth to normal full-term children.

There were also two women who gave birth to full-term infants showing unmistakable eruption of alastrim, although neither mother ever contracted the disease herself, and both had been vaccinated a month or six weeks before delivery.

In the second case the history was as follows: Mother vaccinated on January 10 developed vaccination fever on January 15 and 16. Declares she has not been ill otherwise and shows no pock marks. Child born on February 10 with universal pustular rash in which the pustules are beginning to relax and are umbilicated. Wassermann of mother and child negative. Child died 5 days after birth.

Relation of vaccination to alastrim.

In describing Sanaga pox, Plehn states that vaccination with ordinary calf lymph was successfully performed in thirty-six of forty children six months after recovery from the disease. Ribas, too, found that vaccination of persons who had recovered from alastrim less than a year before was successful in 46 per cent. of the cases; Rudolph in 27 per cent. Aragao vaccinated nineteen persons who had recovered from an attack of alastrim less than a year before and was successful in eleven cases. Rabbits and calves inoculated with alastrim material were in no way immunized against vaccine. Others, too, have been impressed by the fact that alastrim confers little immunity from vaccination with cowpox. Green, experimenting with material from the Australian epidemic, found that inoculation of monkeys and calves produced pustules, but that after recovery these monkeys and calves were susceptible to vaccination with cowpox. His experiments further showed that vaccinia protected against the Australian disease in 25 per cent. of the cases. Variola infection had this effect in 40 per cent. On the other hand, vaccinia protected against variola in 100 per cent., while variola protects against vaccinia in only 50 per cent.

Dr. Moody's study of the relation of alastrim to vaccination in this epidemic gave the following results: 206 cases were observed with regard to the severity of the affection in non-vaccinated and vaccinated cases and these may be recorded in the following table:

	Non-vaccinated.	With scars of successful vaccination.	With history of successful vaccination, but no scars.
Severe Cases	40	16	10
Medium Cases	24	22	22
Mild Cases	14	34	24
Total	78	72	56

He suggests that the proportion of severe cases is higher among the non-vaccinated, but that since none of these people were vaccinated more recently than twenty years ago, not much stress can be laid upon the figures.

In children the disease seems to run a milder course, both as regards constitutional symptoms and rash. Eighty cases were studied, varying in age from sixteen days to thirteen years, and a similar table constructed for them shows the following:

	Non-vaccinated.	With scars of successful vaccination.	With history of successful vaccination, but no scars.
Severe Cases	14	0	2
Medium Cases	10	4	0
Mild Cases	20	22	4
Total	44	26	6

Thus there is evident a general tendency to a milder type, and the effect of successful vaccination is shown in the greater proportion of mild cases as contrasted with the non-vaccinated group. Nevertheless, it is clear that in spite of vaccination, which in these children could not be of very remote date, there were many cases of alastrim. One mother whose child had the disease was vaccinated successfully about the middle of July and returned to the hospital on August 19 with a well-marked vaccination scar and alastrim in the sixth day of the rash. Of fifty-five of the patients actually ill with the disease in Bumper Hall, I found that twenty-four had never been vaccinated, twenty-eight claimed to have been vaccinated in childhood or at school; one, a child with moderate rash, presented an active vaccination pustule hardly healed, while two others had definite vaccination scars. Such evidence, however, is of little value except, perhaps, in the last three, because the statement of the twenty-eight who said they had been vaccinated in childhood was not supported by the presence of a visible scar.

Physicians and medical health officers in Kingston were somewhat divided in their opinion as to the efficacy of vaccination with the standard cowpox vaccine as a protection against alastrim, but those who saw many cases, such as Dr. Murray, felt that the protection was entirely to be relied upon. He had vaccinated more than five hundred contacts among whom not a single case of alastrim arose. Dr. Moody vaccinated twenty persons who had been in contact with the disease without an instance of subsequent infection. Dr. Murray also demonstrated cases of alastrim in the unvaccinated members of some families while the others living in the same house escaped because they had been vaccinated.

It is difficult to form a very precise idea of the efficacy of vaccination in protecting against alastrim, but since among the actual cases of the disease there were so very few that presented indubitable evidence of having been recently vaccinated, and since the escape of those successfully vaccinated is so well attested in large numbers of cases, it seems necessary to believe that vaccination is an extremely effective protection.

Vaccination is employed naturally because it is difficult to draw a sharp line between alastrim and smallpox. An effective protection must be held to be evidence of their extremely close relationship, while any failure to protect might be regarded as an indication that alastrim is a different disease, since the great effectiveness of vaccination as a protection against smallpox is attested by the experience of more than a century, and is absolutely established in spite of the recurrent outcry of ignorant people who attempt to interfere with the use of this great triumph of preventive medicine.

With regard to the effect of vaccination after recovery from alastrim, Dr. Moody makes the following report: Sixty cases were vaccinated during convalescence at periods varying from fifteen days after the onset of the rash up to the twelfth week. Of these, fifteen showed no sign of a " take " in two weeks, and of these fifteen, twelve were revaccinated with three " takes." The method used was that of simple incision through the surface layers of the skin, after which the liquid virus was rubbed in. Of the forty-five who " took " there were none who gave a typical reaction as compared with normal controls vaccinated at the same time with the same batches of lymph. Instead, the incision healed over and no sign of take was visible before the seventh or eighth day, when a few papules were seen in the line of the incision. The papules increased but slowly in size and by the fourteenth day were raised about 2 mm. above the surface of the skin. The lesion ran a very indolent course. No induration or swelling of the arm was noted and in only a few cases were there fever, general malaise, adenitis, or even an areola for more than 0.5 cm. around the lesion. Even in these the symptoms were hardly noticed. On pricking the vesicles a small quantity of clear fluid was obtained. The lesion was multilocular, and when the top of the vesicles was removed it was seen that the base was composed of exuberant granulations rising above the level of the skin. The lesion disappeared, as a rule, in four weeks, and the resulting scar was not the depressed pitted scar of typical vaccine, but a scar slightly raised and subsequently contracting to the general level.

The process differs from that of typical vaccinia, therefore, in the tardy development and slow course of the lesion, the small size of the vesicles, their exuberant base and imperfect umbilication with the atypical scar thus produced, and the insignificance of local and constitutional reaction. The suggestion is made that something capable of causing this reaction exists in the lymph apart from the protective factor, but this can hardly be maintained, since in that case many normal persons unsuccessfully vaccinated ought to develop this peculiar lesion. Something of its appearance can be seen in Fig. 15.

Animal experiments.

With regard to the transmission of these mild diseases to animals, the following may be said: Carini produced Guarnieri bodies in the cornea of rabbits with pus from alastrim, but Rudolph failed in the same experiment. Aragao was able to produce a typical corneal reaction in rabbits with Guarnieri bodies, but could not transfer this effect to a second rabbit. Nor could he produce any lesions in the skin of rabbits or calves by inoculation of the alastrim material. Vorwerk failed to produce any effect by inoculating the contents of a pustule from Sanaga pox in the skin of a calf, and similarly Nägele failed in rabbits and calves with the material from cases of Amaas. Green inoculated the Australian material in monkeys and calves and secured positive results. He inoculated other monkeys and calves with true variola and compared the effects, and states that the Australian disease is different from vaccinia and variola in calves, but very like them in monkeys. Vaccinia in calves fails to protect against inoculation of the Australian disease, and in turn inoculation with the Australian virus fails to protect against either vaccinia or variola.

We inoculated rabbits with blood from cases of alastrim in the preeruptive febrile stage, thinking that at that period the infective agent might be present in the circulation. Blood was injected intravenously, into the testicle and allowed to flow over the scarified cornea. There were no results. Other rabbits were inoculated in scarifications in the skin and in the cornea with the contents of pustules from typical cases, but although they were most carefully watched for many days, the scarifications healed without the formation of any recognizable lesion. The corneas never showed the slightest opacity, nor any translucent nodules such as are described by Paul, although carefully examined with a lens. The scarifications healed quickly and the cornea returned to normal.

Three calves were inoculated in the skin of the flank with material freshly obtained from the skin lesions of typical cases of alastrim, but these, too, failed to develop any vesicle or pustule, and the scarifications healed smoothly at once.

We were thus entirely unsuccessful in transmitting the disease, in however localized a form, to rabbits or calves.

It is pretty generally agreed now that the so-called Guarnieri bodies which occur in the epithelial cells about a smallpox pustule, and which appear in much greater numbers in the epithelial cells of the rabbit's cornea when either vaccinia or variola is inoculated, are fairly specific in their relation to these diseases, and that they do not represent the aetiological agent, but rather the reaction of the cell to its presence.

v. Prowazek, Paschen, Hallenberger and others have written much recently concerning minute bodies which are present in the lesions of vaccinia and variola and are also found, according to Aragao, in alastrim. v. Prowazek, their discoverer, regards them as the actual cause of these diseases. They can pass through a Berkefeld filter which removes bacteria, and the filtrate will produce the typical corneal lesion in rabbits. They can be concentrated by passing the filtrate through a colloid filter made of agar after which the second filtrate is inert. Hallenberger describes the morphology of these minute bodies in detail and shows them as extremely small round, dumbbell-shaped or dividing bodies, sometimes unequally parted so as to look like an exclamation mark. They do not move except to assume a slight dancing motion; they are not stained by ordinary bacterial stains, but can be colored by Giemsa's stain, although Aragao states that their characteristic staining is only to be attained by the use of Löffler's mordant.

We were able to demonstrate these minute bodies by dark-field illumination in the fresh material from the alastrim pocks, where they appear as very numerous, extremely minute, rather refractive particles in fairly active Brownian motion. When this material was passed through a porcelain filter the same particles were still to be seen. They were inoculated into the skin and cornea of a rabbit, but with no result.

Very few of the proofs ordinarily demanded to show the aetiological relation of an organism to a disease have been satisfied in this case. These nondescript minute granules stain rather more specifically than other granules; they are present in great numbers in smallpox and vaccinia pustules and in the epithelial cells. They go through a porcelain filter and the filtrate, whether on account of their presence or that of some other living organism, produces a characteristic corneal lesion. When they are filtered out by the colloid filter, whether alone or in company with something else, the filtrate no longer produces the corneal lesion.

In the case of the alastrim material our uniform failure to produce any lesions made us incapable of adding any evidence for or against these claims.

Discussion and conclusions.

We are still in the ignominious position of being unable to state with absolute certainty the relation between smallpox and cowpox or vaccinia. This uncertainty must continue until the organisms concerned are isolated in pure culture so that their biological characters may be compared, just as types of pneumonia or dysentery could never have been distinguished without the study of pure cultures of the bacteria. The literature which extends over more than one hundred years is full of arguments based upon inoculation experiments, but it remains clear that it is difficult to transmit smallpox to calves, that it has usually been done in vaccine institutes where there was a chance of the development of cowpox, and that in this apparently simple question uncertainty persists. The reasoning in all this literature seems extremely loose and the dogmatic conclusion found in most textbooks, to the effect that vaccinia is merely smallpox attenuated and permanently altered by passage through calves, unjustified. All this literature is reviewed by Tomarkin and Carriere and by many others. It is mentioned here without any intention of discussing it, merely to show that it is equally difficult to discuss the relations between smallpox and alastrim. No definite and final statement of their relation seems possible until the causative organism is isolated in pure culture from typical cases of each disease and compared.

The immunity conferred by an attack of one of these infections or by the inoculation of the virus to produce a local lesion, while of enormous practical value in the prevention of disease, is not a sharp enough test for the separate identity of these closely related diseases.

Vaccination almost absolutely prevents infection with smallpox, but a mild smallpox or varioloid may sometimes occur in successfully vaccinated persons. Vaccination may sometimes be successfully performed after recovery from smallpox.

Vaccination apparently afforded a powerful protection against alastrim, but there were many cases in persons who had been successfully vaccinated, and successful, if atypical, vaccination was possible after recovery from alastrim. These occasional departures from strict reciprocal protection were probably more numerous between vaccinia and alastrim than between vaccinia and smallpox, but this questionable difference is not a sufficient basis for a sharp distinction between smallpox and alastrim. Dr. Deeks tells me of two cases in which alastrim occurred in persons who had recovered from true smallpox.

Cross complement fixation carried out by Kolmer and others showed that antibodies arise in the serum of vaccinated animals and of persons who have recovered from smallpox, but that these antibodies are in both cases capable of combining with the antigen (contents of pustules) derived from both smallpox and vaccinia. This shows a close relation between these conditions, and probably would show a similar close relation to alastrim. The method seems useless, however, in an attempt to show a distinction between alastrim and true smallpox, although there might be quantitative differences.

One of us (L. M. M.) has made four tests by vaccination of the production of an allergic reaction in persons who had recently recovered from alastrim, using as control normal persons who had not had the disease, but who had been recently vaccinated. There was absolutely no allergic reaction in any of these cases, of whom two were white people, although it was well defined in the recently vaccinated controls. In three of the alastrim patients there afterwards developed, however, the indolent vaccine lesion described above.

The evidence is scarcely sufficient to warrant the statement that alastrim is a disease quite distinct from true smallpox. The effort to distinguish them is the more difficult on account of the extraordinary variation in severity in what has in various epidemics been regarded as true smallpox. Mild cases of smallpox often show, however, only a few scattered pocks and a slight fever, while in alastrim the initial fever is high and the body is covered with pocks often broadly confluent, and yet the patients are not very sick, the liability to secondary infection is slight and the mortality trifling.

Clinically and histologically the eruption is somewhat different from that in smallpox in that early umbilication is lacking. Inoculation experiments in animals failed in our hands, but the results of others suggest differences between alastrim and smallpox. Although there are very few recorded instances of the transportation of this disease to other climates, we feel that it would probably maintain its identity in any climate.

The disease is as easily communicable as the most severe smallpox, but its spread has been restricted by prompt vaccination and revaccination. No consideration could excuse neglect of this preventive measure or of the proper quarantining, even though the death rate is low.

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EXPLANATION OF PLATES.

Plate XII.

Fig. 1. *D. H.* 8th day of rash.

Fig. 2. *J. M.* 8th day of rash, 11th day of disease.

Plate XIII.

Fig. 3. *Hall.* 3d day of rash, 5th day of disease. Eruption confluent on forehead.

Fig. 4. *Hall.* 5th day of rash.

Plate XIV.

Fig. 5. *Hall.* 7th day of rash. Patient ill and photographed in bed.

Plate XV.

Fig. 6. *Hall.* 9th day of rash.

Plate XVI.

Fig. 7. *Mrs. W.* 9th day of rash, 11th day of disease. Many of the pustules show central desiccation.

Fig. 8. *D. R.* 4th week of disease. Crusts still adhere to face, but the body shows smooth depigmented areas with zones of pigmentation.

Plate XVII.

Fig. 9. *G. W.* 4 months after severe attack of alastrim. Pitting is practically absent and only faded areas of pigmentation remain.

Plate XVIII.

Fig. 10. *Hall.* Papule at 3d day of rash showing infiltration about the vessels of the corium and balloon-like degeneration of the epithelium.

Fig. 11. *Hall.* Vesicle in 3d day of rash, near a hair follicle, with hyalinization of overlying epithelium.

Plate XIX.

Fig. 12. *Williams.* Margin of a pock at 9th day of the rash. Compare Fig. 7 from the same patient at the time of excision of this pustule.

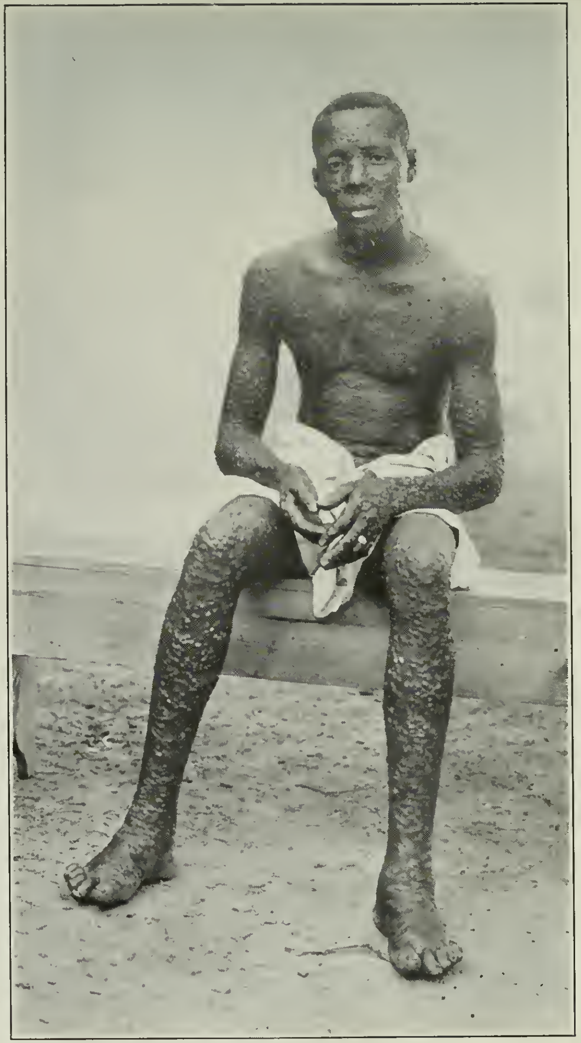

Fig. 1.

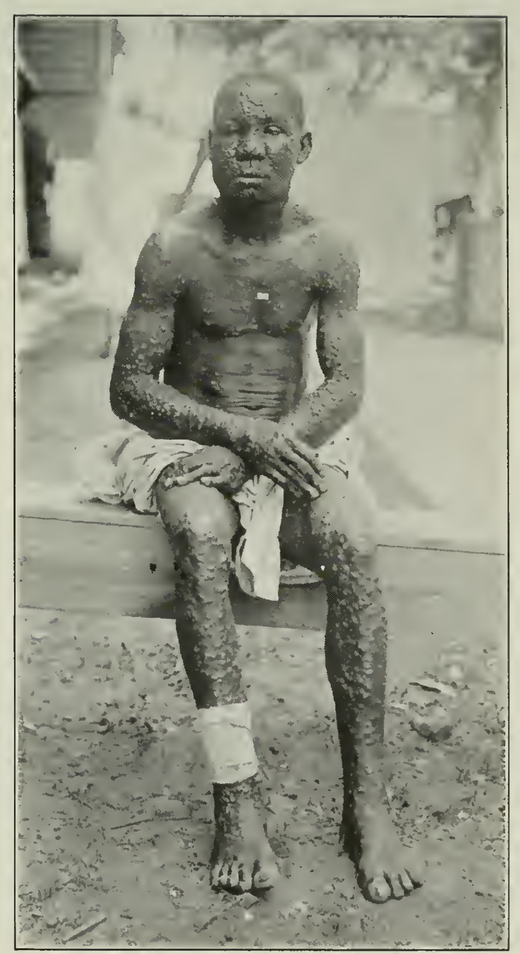

Fig. 2.

PLATE XII.

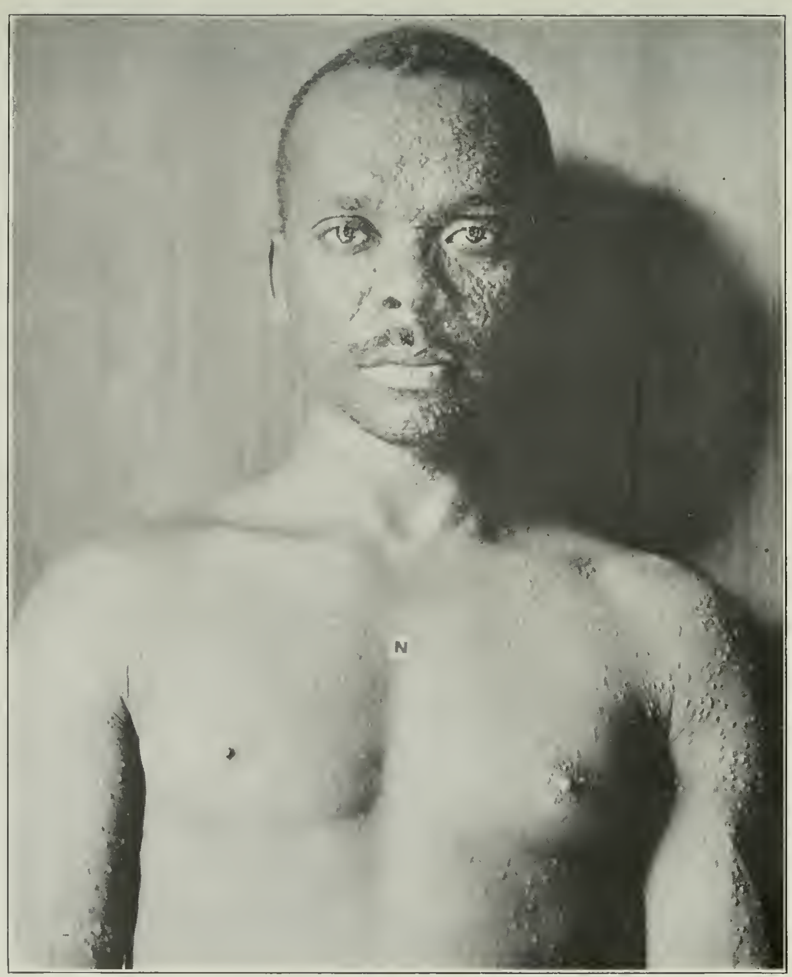

Fig. 3.

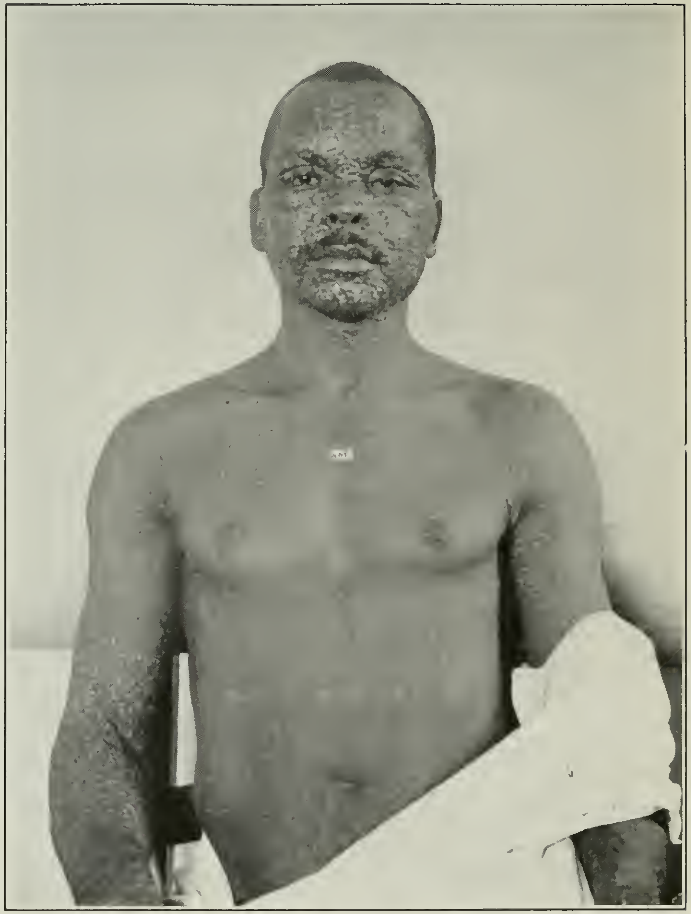

Fig. 4.

PLATE XIII.

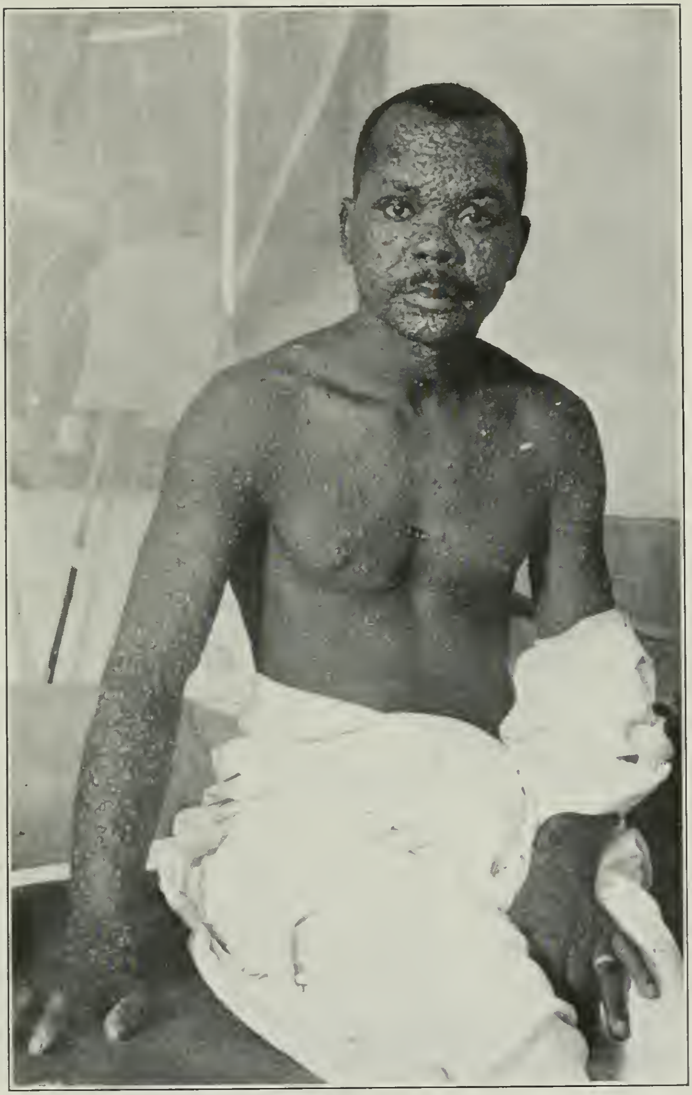

Fig. 5.

PLATE XIV.

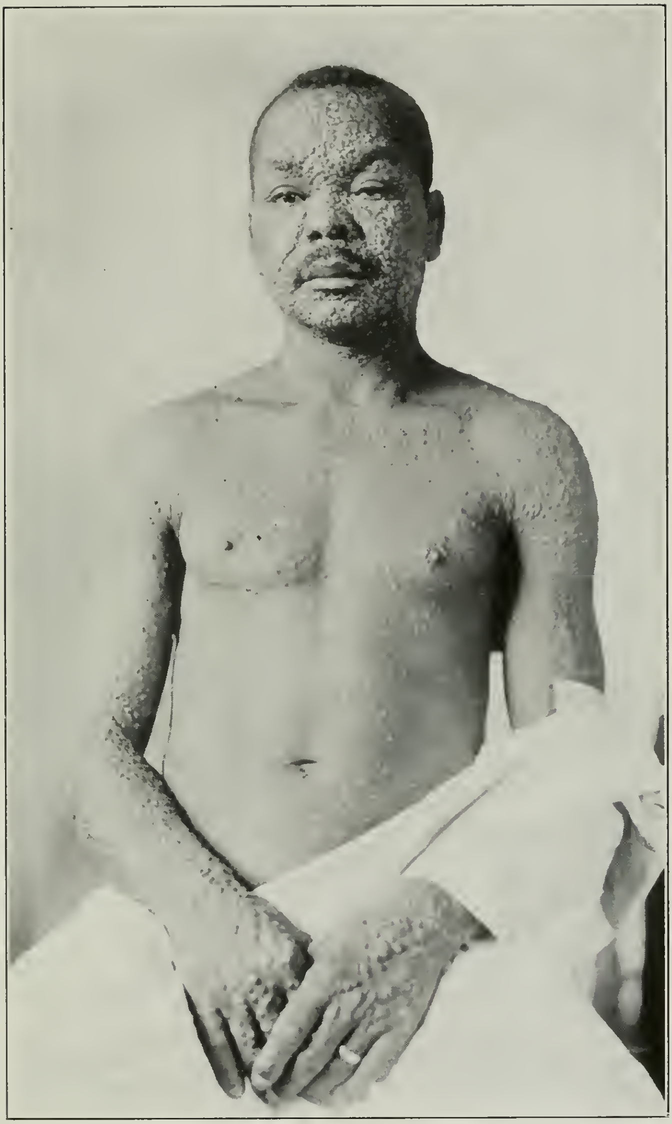

Fig. 6.

PLATE XV.

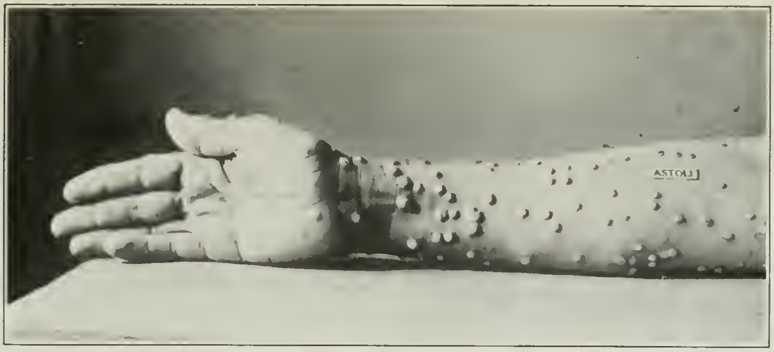

Fig. 7.

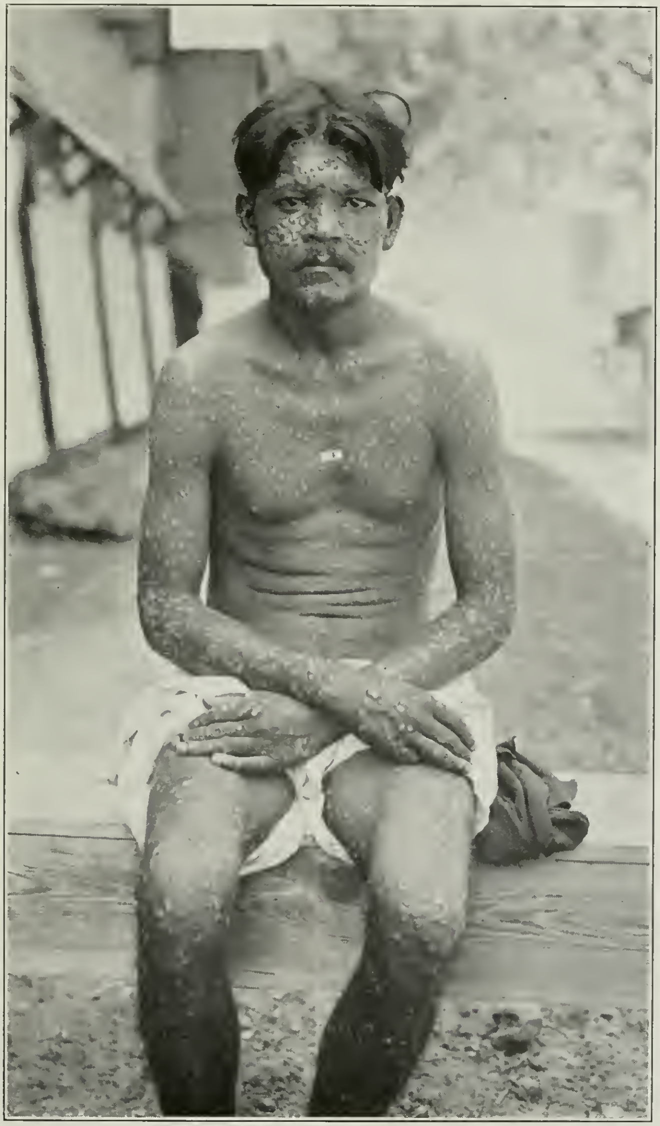

Fig. 8.

PLATE XVI.

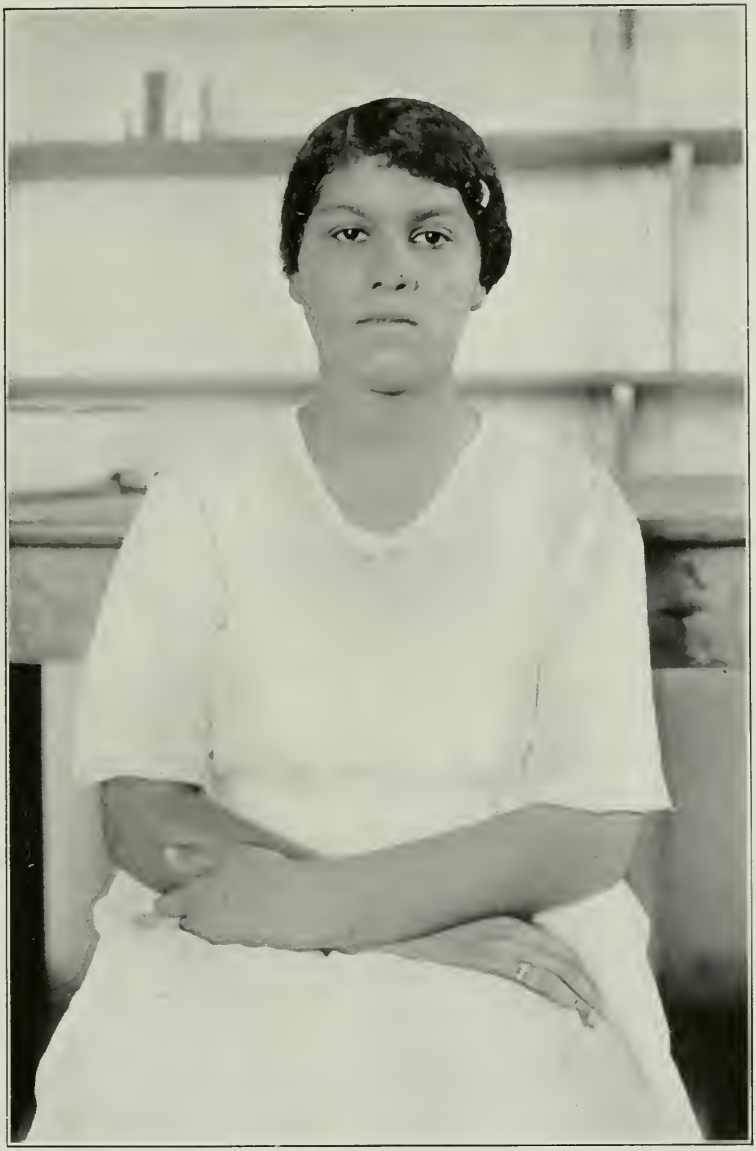

Fig. 9.

PLATE XVII.

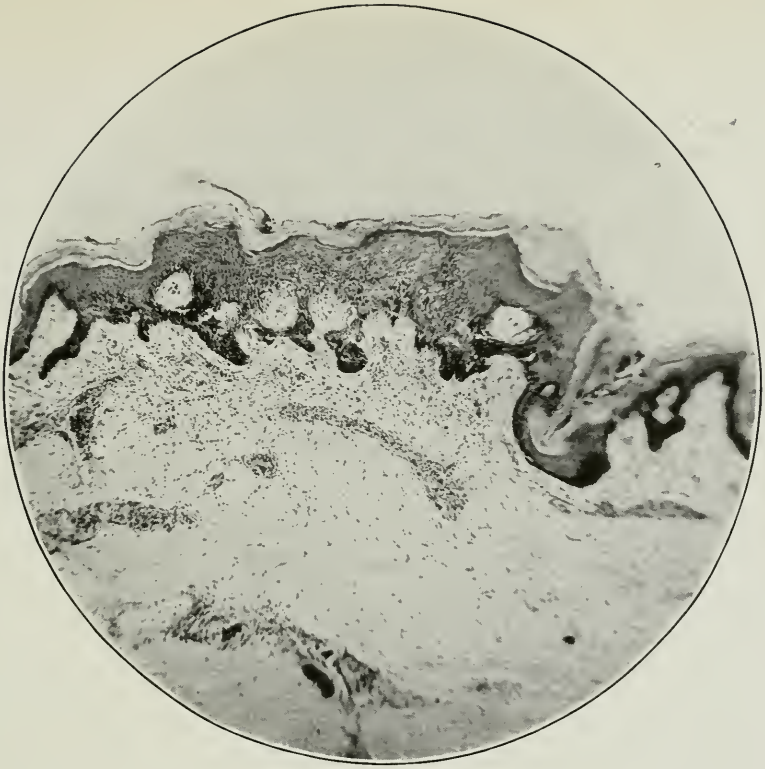

Fig. 10.

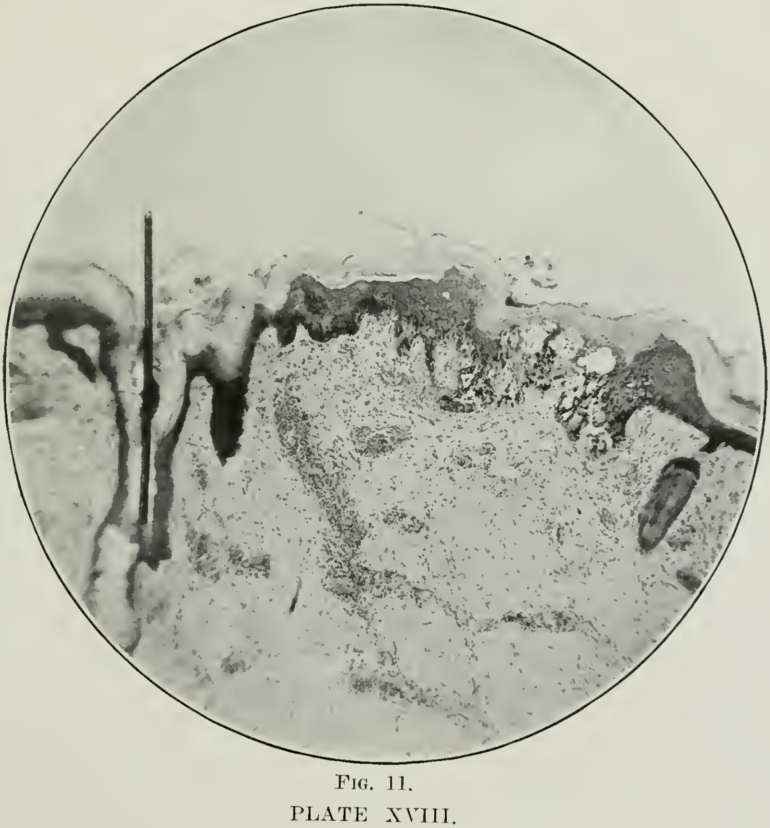

Fig. 11.

PLATE XVIII.

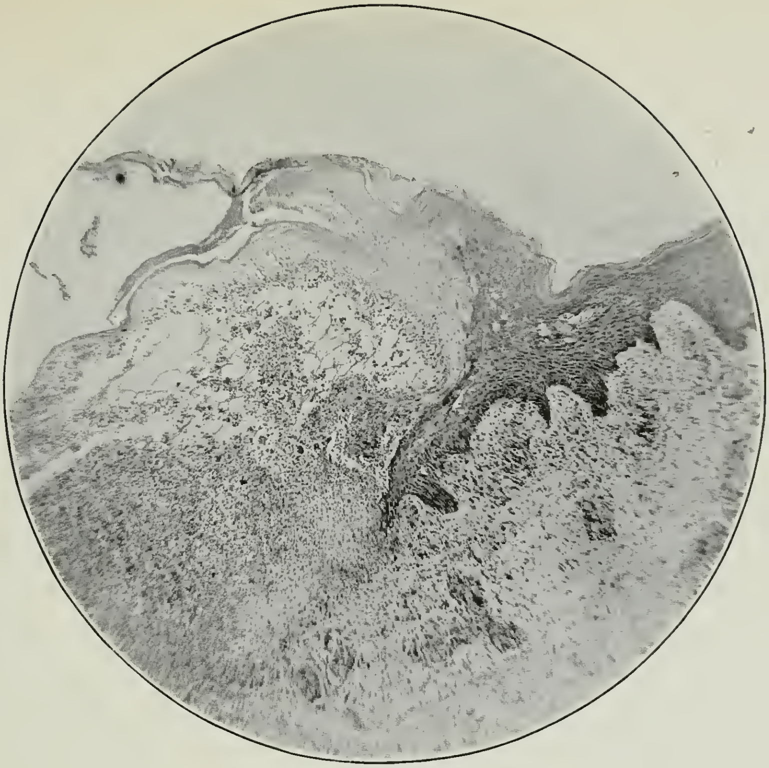

Fig. 12.

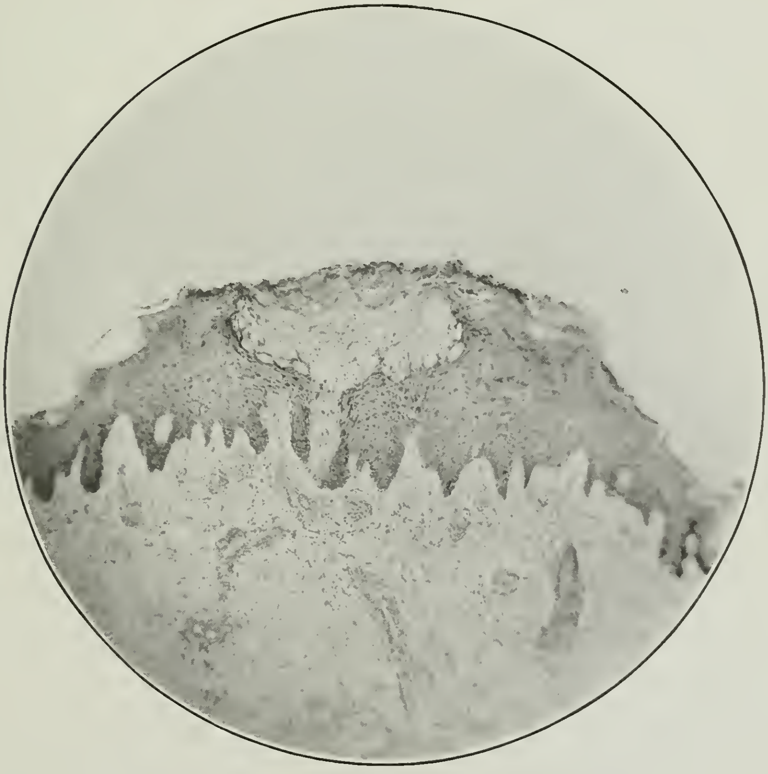

Fig. 13.

PLATE XIX.

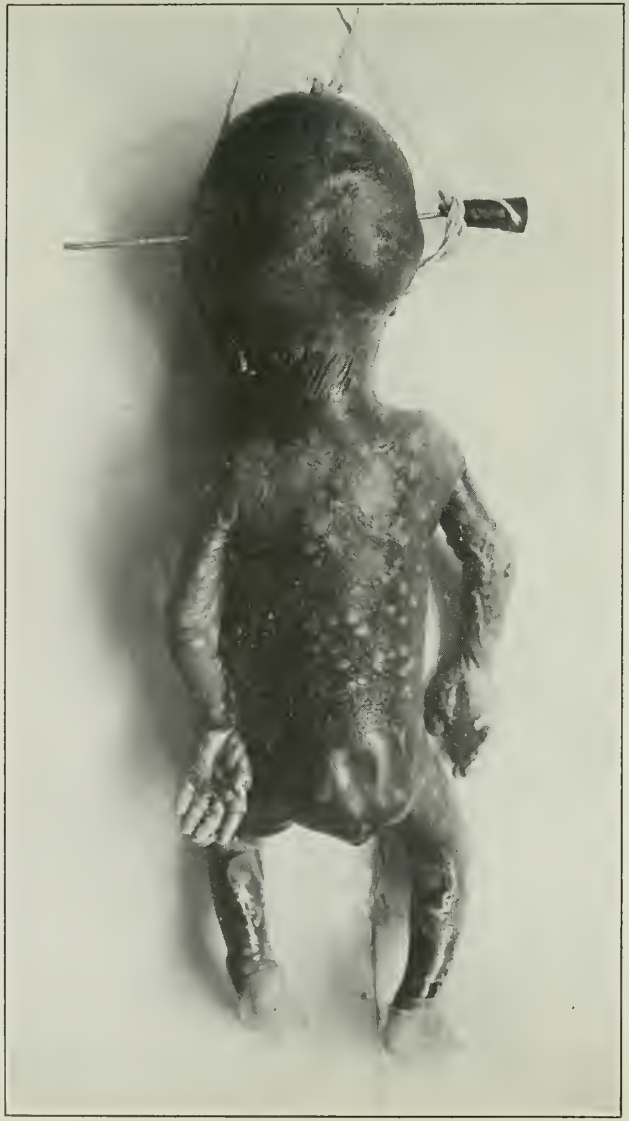

Fig. 14.

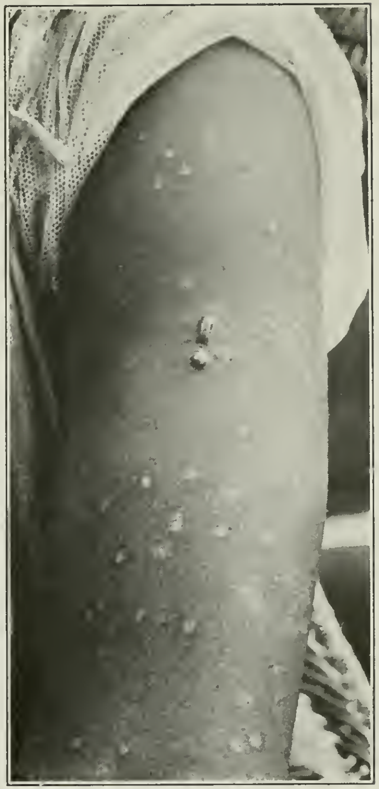

Fig. 15.

PLATE XX.

FIG. 13. *Burton.* Vesicle in 3d day of rash showing infiltration of the corium and projection of a papilla into the edge of the cavity in the epithelium.

PLATE XX.

FIG. 14. Macerated foetus with distinct marks of alastrim pocks upon the skin.

FIG. 15. Vaccination of patient at the 13th day after the onset of symptoms of alastrim. Photograph taken 10 days after vaccination shows the superficial marks left by the alastrim at about the 20th day and the atypical vaccine pustules.

THE HUMAN TRICHOMONAS IN NORTH CHINA.*

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Peking, China.

PLATE XXI.

(Received for publication April 18th, 1921.)

Records of *Trichomonas hominis (intestinalis)* are common, but the species is not well known, nor is its pathogenicity well understood. It has usually been found in liquid stools in cases of diarrhoea and has been reported from practically all localities where careful fecal examinations have been made. In clinical laboratories in China where this parasite has been differentiated from other protozoan infestations it has an infection incidence of from 0.5 per cent. (Nanking, Chefoo) to 0.14 per cent. (Shanghai). This incidence is relatively smaller than Wenyon and O'Connor's figures (1917) for British troops in Egypt (3.0 per cent. for all cases examined), but the latter records were from selected cases, so that the absolute infection incidence may actually be higher in China.

Protozoologists have definitely accepted Davaine's variety B of "*Cercomonas hominis*" as the original type of the species. More recent investigators have distinguished from 3 to 5 flagella, an undulating membrane with marginal filament, an axostyle, a parabasal body, a blepharoplast and a cytostome. The actual descriptions of these organelles have, however, been extremely variable and confusing. Since Wenyon's figures on "*Trichomonas intestinalis*" from the mouse (1907) can not be regarded as identical with the human form, no satisfactory figures on the species have been published.

The material on which this paper is based was secured from samples of human feces submitted to me by the medical staff of the Peking Union Medical College Hospital for examination for amoebae. The stool was liquid, of a greenish-black color and disagreeable odor. Smears under cover glass showed a large number of extremely active *Entamoeba histolytica* with ingested red-cells and numerous *Tricho-*

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* Contribution from the Department of Pathology, Peking Union Medical College. Read before the Society of Laboratory and Clinical Medicine, March 16, 1921.

monas hominis. There were also eggs of *Taenia saginata.* The protoplasm of the trichomonad was so mobile that it simulated the movements of the amoeba to a high degree, but, like Ohira and Noguchi's material (1917), it had no true, food-ingesting pseudopodia. Three days later the amoebae were not found in the stool, but daily examinations for the flagellate were positive until the eleventh day. The organism did not appear again during the period of observation.

DESCRIPTION OF THE ORGANISM.

Under high power of the microscope the living *Trichomonas hominis* is extremely motile. This activity is due to (1) the extreme plasticity of the protoplasm, resulting in a free amoeboid movement of the whole organism, and (2) the combined action of the anterior flagella and the undulating membrane. This results in a rapid forward movement of the organism even against a heavy current. All living specimens which I have observed were positively rheotactic. This orientation against the current is facilitated by the long stiff axostyle which acts as a brace, holding the ground previously gained, while allowing the organism free play in further forward movements. This axostyle is placed somewhat excentric; it lies nearer the cytostome than the undulating membrane. As the parasite moves forward in a rapid dextral spiral motion, the axostyle is frequently thrown far out to the side, but always remains stiff. Kofoid and Swezy suggest (1915) that this organelle is probably a differentiated flagellum.

At times of rapid forward movement the shape of *T. hominis* is elongate-pyriform. At times of quiet feeding the shape is more rotund. Under all conditions in the living organism the axostyle extends far posteriad, 4 to 8 μ beyond the limits of the protoplasm. In fixed smears it is less conspicuous.

At body temperature the parasites remain active in the stool for only a few hours. At 22° to 25° C. the organisms were living after 20 hours, and some survived for 48 hours. At 37° C. rapid encystment was observed. They also remained motile in weak iodine dissolved in normal saline, for several hours.

Preparations for preservation were fixed in Gilson's fluid. After careful washing, they were transferred through grades of alcohol to 2 per cent. iodine in 70 per cent. alcohol. They were then mordanted in tannic acid-fuchsin for one minute, stained in saturated fuchsin in aniline water and partially destained. They were then treated in the usual way with Heidenhain's Iron-Haematoxylin, cleared and mounted. While it is not as easy to study stained preparations of *T. hominis* as those of related species, essential points can be made out with certainty.

Confined specimens of living *Trichomonas hominis* under observation have an average measurement of 12 μ in length by 7 μ in width, while the preserved specimens measure 10μ in length by 5μ in width. The portion of the axostyle behind the body has an additional length of 6 μ in the living organism, but is shorter in preserved material. When at rest the living trichomonad tapers equally at both ends, but in active forms the posterior end is much the larger. The nucleus is a large spherical body, varying from 1.5 to 3.5 μ in diameter. The protoplasm is usually filled with numerous food and, at times, pulsating-vacuoles. The pellicle is thin, but tough. The cytostome is located to the right of the nucleus and has a sinistral torsion, so that the movement of the animal is clockwise. I have frequently observed food vacuoles emptying their contents to the outside at a constant position in the left posterior margin just under the undulating membrane. This suggests the possibility of a definite anal opening in the species (see Figs. 4, 7).

The blepharoplast is a single spherical body which at times (Fig. 1) is large and composed of discrete granules, at other times drawn out into a dumb-bell shape (Fig. 7). It is the seat of origin of (1) the anterior flagella, (2) the marginal filament and (3) the costa or parabasal body, and is connected by one fibril (the rhizoplast) both to the nucleus (Figs. 8, 11, 16) and the axostyle.

The anterior flagella in the material examined are three in number and of approximately equal length. In over one hundred stained specimens where the flagella were clearly visible I found no variation in the number. They are much more delicate than those of other species which I have studied, a fact which may account in part for the difficulty which observers have experienced in counting the flagella. The undulating membrane lies on the left side slightly dorsad. It is a broad band that extends almost to the posterior margin of the animal. The marginal filament is slightly heavier than the anterior flagella. In my material it does not extend as a free flagellum beyond the limits of the undulating membrane. Myomeric differentiation of the undulating membrane is clearly distinguishable in the living mounts. The costa arises in the blepharoplast and roughly parallels the undulating membrane. It is not surrounded by deposits of chromatinic granules, as described for several trichomonad species, but is itself at times (Figs. 9, 10, 11, 13) broken up into a row of such bodies. The axostyle is a long straight hyaline rod extending from near the blepharoplast far beyond the posterior limit of the body. It is not usually distorted by the movement of the living organism, but is occasionally shrunken and bent in fixed smears (Fig. 11). Even under these circumstances it does not contract lengthwise in proportion to the protoplasm. It is studied without difficulty in the living animal, but resists ordinary staining methods.

As so-called encystment approaches, the undulating membrane first begins to contract and later (Figs. 14, 15) the anterior flagella become crimped. At the same time a large vacuolate space appears around the blepharoplast, and later around the nucleus. Finally the membrane and flagella become indistinguishable and a simple ovoid form results (Fig. 16). This seems to be a degenerate form rather than a true cyst. In fact, true cyst formation in this species has not been satisfactorily demonstrated.

I have not observed fission, although I have seen large vegetative individuals suggesting a pre-fission stage. The protoplasm is frequently filled with pulsating vacuoles (Fig. 8) and at times contains large masses of food, so these individuals appear almost opaque. The specimens apparently lack chromatinic granules around the cytostome and nucleus, and along the axostyle and costa.

Discussion.

The descriptions of *Trichomonas hominis* by various investigators have shown great variation. A comparison shows that size is obviously not diagnostic, since recorded measurements vary from 4 to 30 μ in length by 2 to 23 μ in width. Certain workers figure the species with an axostyle not extending beyond the protoplasm (Prowazek, 1912), while Wenyon and O'Connor (1917) figure a long sharp rod. Since Wenyon's work on Trichomonas of the mouse probably refers to a different species, there is agreement on the blepharoplast being a single body and on the absence of chromatinic granules throughout the body. The costa is likewise evidently not an organelle which can be regarded as diagnostic, since it shows practically no differentiation. Furthermore, Rodenwaldt (1912) and Wenyon and O'Connor (1917) figure individuals having a marginal filament with a free distal end, while Neumann and Mayer (1914, Tab. 1, Figs. 24, 25) show specimens without this posterior free end. Ohira and Noguchi (1917) have sometimes seen a short free end. My material shows no free distal end. As regards the anterior flagella, Rodenwaldt (1912), Doflein (1916), Fantham (1916) and Castellani and Chalmers (1919) describe three, but while Wenyon and O'Connor (1917) have seen individuals both with three and with five flagella, the majority of their specimens contained four. After an examination of his own material, as well as that of Lynch, Walker and Haughwout, Kofoid (1920) has come to regard four flagella as typical, and on this basis has predicated that the genus *Trichomonas* must be regarded as a tetraflagellate group. He has proposed a new genus, *Tritrichomonas,* for the triflagellate species. He states, however (p. 159), that "there is so much discrepancy in the results of different investigators as to the number of flagella that one can not be dogmatic and deny the existence of species in man with only three flagella."

My studies on material in which three anterior flagella seems to be the normal number brings up anew the diagnostic value of the anterior flagella.

In my opinion the species *Trichomonas hominis* possibly includes all trichomonad forms described from the human intestine that have definitely been shown to possess (1) an axostyle, (2) a true undulating membrane with marginal filament whether the latter has a short free-end or no free-end at all, (3) a costa paralleling the undulating membrane, whether entire or broken up into a row of chromatinic granules, (4) a cytostome situated opposite the membrane, (5) a single blepharoplast as the center of the neuromotor apparatus, and (6) anterior flagella varying in number (three to five in described species), but tending to be constant in particular races. These flagella may be of equal or unequal lengths. In addition, the species lacks the chromatinic granules around the axostyle, costa, nucleus and cytostome. These granules have a characteristic distribution in other species of trichomonads, but have never been reported for the human form. It seems quite probable, likewise that the several forms reported for the human intestine may be the same species, but *Trichomonas vaginalis* differs both in morphological and biological characters.

CULTURE IN VITRO AND INOCULATION EXPERIMENTS.

Lynch (1915) was the first to report a successful cultivation of human trichomonads. He placed the organisms obtained from cases of catarrhal vaginitis and acute gingivitis in neutral and acid bouillon at 30° C., and by subculturing was able to grow several generations. Later Ohira and Noguchi (1917) used a solution of ascitic fluid and

Ringer's solution half and half, culturing the forms from the tartar of the teeth at 37° C., and subculturing for over 20 generations. At a lower temperature (23–27°) the multiplication was less rapid, but on the whole equally satisfactory. Their medium was more satisfactory than that of Lynch, because its natural alkalinity prevented an overgrowth of bacteria. Recently Pringault (1920) has modified the Ohira and Noguchi medium by reducing by half all of the salt proportions of the Ringer's ingredient. In this way he has secured multiplication in 8 hours. Boyd (1919), using a fecal suspension, has kept cultures alive up to sixty-five days.

Lynch (1915) was able to infect a white rabbit with fecal trichomonads by injection per rectum. Boyd (1919) infected a rat per os. After recovering trichomonads from the feces and culturing them he found them to be identical with his original culture forms. Escomel (1913) claims to have infected the rabbit, guinea pig, cat and dog. On the other hand Pringault (1920) had negative results from inoculations of the cat, guinea pig, rabbit and white rat per os and of a kitten per rectum. Evidently much remains to be learned regarding the exact methods of infection in trichomonadiasis.

PATHOGENICITY OF *Trichomonas hominis*.

There exists great diversity of opinion as to whether *Trichomonas hominis* plays a pathogenic rôle in the human intestine. On the one hand Minchin (1912) states that "the common intestinal flagellate belonging to the genus *Trichomonas* and other genera are . . . not to be regarded as true parasites in any sense of the word." On the other hand Rhamy and Metts (1916) believe them to be the causal agents of the dysenteric diarrhoea with which they are associated, and cite several fatal cases as instances. Likewise Escomel (1913) records 152 cases of so-called pure *Trichomonas* dysentery from Arequipa, Peru (1898). He attributed the infection to impure drinking water, since the dysentery abated with the purification of the water supply. Haughwout (1918) refers to the possible effects of the organism as follows:

"1. The production of antigrowth vitamines or growth-inhibiting substances. . . .

"2. The production of substances directly toxic.

"3. Unfavorable effects upon the host through the liberation of the products of metabolism of the parasite.

"4. Mechanical irritation of mucous surfaces by the parasite when present in large numbers.

"5. Interference with absorption in the intestine through the adherence of large numbers of parasites to the surface of the epithelium, as in the case of *Lamblia*.

"6. Actual invasion and destruction of the tissues with all its concomitants and sequelae."

An analysis of this list of possible indictments taken hand in hand with reported cases shows that in some cases the indictments may hold true, but in others the counts cannot be more than partially sustained. Wenyon and O'Connor (1917) believe it to be "quite possible that sometimes a flagellate produces symptoms of diarrhoea, resulting from an irritation produced by large numbers of the organisms, but . . . are convinced that in the majority of cases of diarrhoea its presence is only accidental, and that it is fixed upon as the cause of trouble because it happens to be the most conspicuous organism in the stool."

This statement apparently holds for the case from which I obtained the material for this study. The condition of the patient did not improve after the flagellates became negative in the stool. The structure and use of the axostyle is such, however, as to make the parasite a possible physical irritant to the mucosa. As Haughwout suggests (1918) different degrees of pathogenicity reported for this flagellate may be due to different degrees of virulence. On the other hand the unsatisfactory description of the species may have led to error regarding the flagellate under consideration, if there really are more species involved.

In view of the common occurrence of *Trichomonas hominis* along with *Entamoeba histolytica* it seems advisable to search carefully for living or encysted amoebae, where the trichomonads are found, for a symbiotic relation may possibly exist between these two organisms.

Summary.

1. In spite of the large number of records of *Trichomonas hominis* descriptions of the parasite have in many cases been vague and on the whole conflicting.

2. Workers have been inclined to differentiate trichomonad forms into genera on the basis of the number of anterior flagella. Further study may show that these groups are merely varieties or races of one species, *Trichomonas hominis*.

3. *Trichomonas hominis* has been cultured in vitro by several investigators and has probably been inoculated into experimental animals.

4. Reports on the pathogenicity of *Trichomonas hominis* range from a non-pathogenic rôle to one causing fatal dysentery. In the majority of cases no actual proof of the etiological connection between *Trichomonas hominis* and diarrhoea and dysentery has been produced, although it is frequently concerned and may even produce a mechanical irritation of the mucosa of the host.

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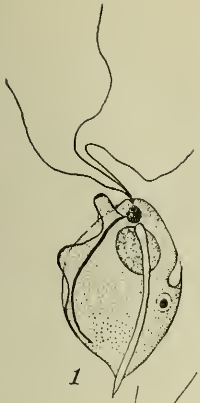
EXPLANATION OF PLATE XXI.

Figs. 1–16, showing characteristic specimens of preserved material of *Trichomonas hominis.*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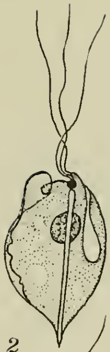
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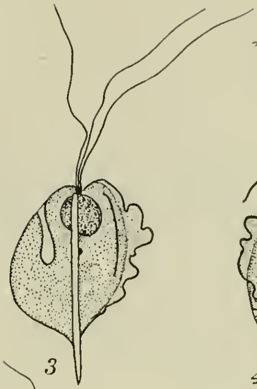
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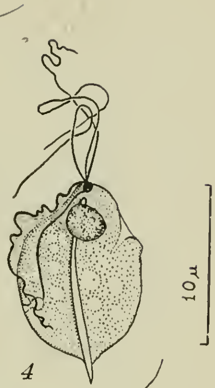

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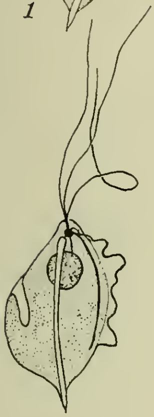
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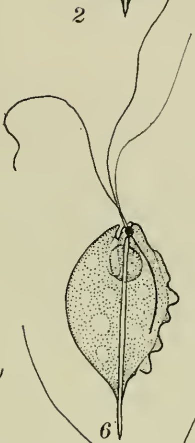
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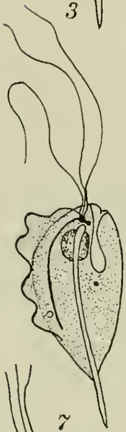
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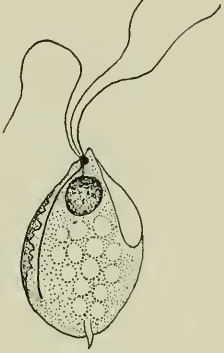
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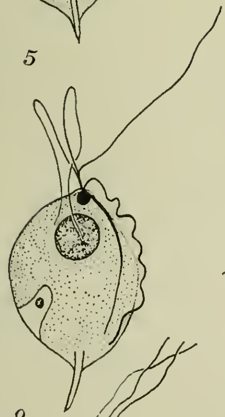
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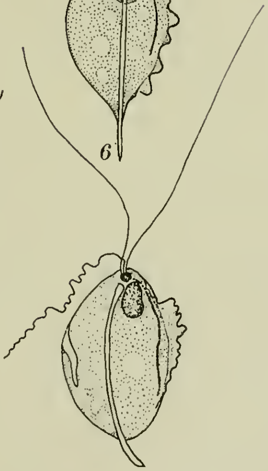
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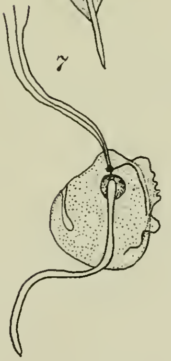
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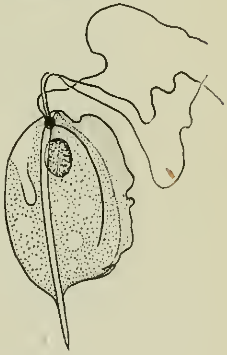
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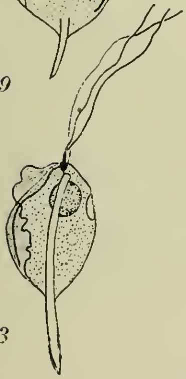
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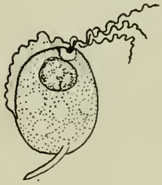
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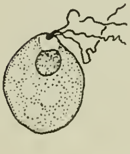
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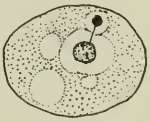
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PLATE XXI.

BIOMETRIC DATA ON INFANT MORTALITY IN THE UNITED STATES BIRTH REGISTRATION AREA, 1915–1918.*

By RAYMOND PEARL.

(Received for publication May 20th, 1921.)

Introduction.

Until recently it has been impossible to discuss on a satisfactory basis the infant mortality of any considerable portion of the United States. This difficulty has arisen from the fact that except in a few localities, notably some of the New England States, there has been in the past no adequate system of birth registration. The most accurate practical method of stating the force of infant mortality is to relate the number of deaths of infants under one year of age in a given time unit to the number born in the same time unit. The expression of infant mortality rates as deaths under one year per thousand births in the same time interval is not entirely accurate in theory. Instead of using the "same time interval" one should use the births of an "equivalent" year to get results of the highest accuracy, as has been pointed out by Knibs (1917). But to a first approximation the ordinary method of using the same time interval for both the births and the deaths is sufficiently accurate.

One must have accurate birth statistics before infant mortality can be adequately discussed. Theoretically, of course, one might use in census years the count of the population of infants under one year of age as the denominator of the mortality rate fraction or the estimated population for the same age group in inter-censal years. Such procedure has, however, never been regarded by vital statisticians as entirely satisfactory because of the large errors which are known to inhere in counts of the infant population. Recently Crum (1920) has, apparently on the somewhat dubious ground of immediate convenience and expediency, used for the study of infant mortality, an "infant mortality index'' which is defined as ''the infant mortality per 1000 of estimated population under one year of age.'' Regarding this index he says:

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* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 39. A brief preliminary report of this work has been published elsewhere (cf., Pearl, R., 1920 and 1921).

''Infant mortality rate has come to mean infant deaths per 1000 births, the deaths and births being for the same period—week, month or year, as the case may be. For comparative purposes the 'index' as here determined is probably as accurate, if not more so, that the 'rate' as births are nowhere so completely reported as deaths and the variation in the completeness of birth reporting is considerable, even in the large American cities.''

This would seem to be distinctly a retrograde step in biostatistical research. Furthermore one cannot agree that this index is ''probably as accurate, if not more so,'' than the usual rate, particularly in view of the well known inaccuracy of even total population estimates towards the ends of inter-censal periods, which is just the point at which Crum is working. We may well expect, in view of the fact that the general United States population curve has passed its point of inflection (cf. Pearl and Reed, 1920) that for some time population estimated by the usual methods will be particularly inaccurate. Again, in such a case as Crum's usage, the estimate is not of the total population, but of one small age group. Also during the war period industrial movements connected with the war profoundly disturbed the normal population growth of large cities. Altogether it is believed that the safest ground is to stick to the normal infant mortality rate per 1000 living births.

It is a matter of great satisfaction that at last there is well established a Birth Registration Area for the United States, and five annual reports on Birth Statistics of this area have been issued to date by the Census Bureau. We are well embarked now on the policy of adequate birth statistics for the country, and unquestionably within a comparatively few years the Birth Registration Area will cover the major portion of the country as the Death Registration Area now does. In the short period since the Birth Registration Area has been established its growth in extent has been gratifyingly rapid. The first report on birth statistics for the year 1915 comprised data from an area including approximately only 31 per cent. of the population of the country. The 1918 birth statistics report gives data from an area including 53 per cent. of the population. This furnishes a sufficient volume of material so that one may begin the mathematical analysis of some of the problems of infant mortality with some assurance of reaching valid conclusions.

The purpose of the present paper is primarily to present the facts, with some simple mathematical analysis, of *variation* in rate of infant mortality in different geographic or demographic units of the population. These facts of variation make it clearer where the fundamental administrative problems of control of infant mortality lie than perhaps could be done in any other way. The first step in the solution of any problem is obviously a clear definition of the problem itself. We shall see, as we pass from city to city, town to town, or rural country to rural country, that the rate of infant mortality varies greatly. In the hypothetical community where the most perfect administrative control over infant mortality possible or conceivable had been attained this variation would to a considerable extent disappear, the only residue of diversity between communities in respect of infant mortality being such as arose either (1) purely by the operation of chance, that is, from random sampling, and (2) from the racial composition of the several populations, and (3) from fundamentally uncontrollable environmental differences, such as climate, soil, etc. Now with the actually existing condition of variation between different communities in respect of infant mortality, it is obvious that there must be particulate and presumably in large degree determinable reasons for each particular difference which exists. Equally obviously, before administrative control can effectively wipe out these mortality differences and get all communities at or near the level of the lowest, we must know something about the determining causes upon which they depend. Operating on a basis largely of empiricism and *a priori* reasoning, efforts to reduce infant mortality have in the past been attempted with considerable success. Also, with the advance of general sanitation the death-rate under one year of age has fallen enormously. Greenwood (1912) quotes some interesting figures on the point from Farr, which we may well reproduce here to show how enormous has been the improvement.

TABLE 1.

Showing the reduction in the mortality of infancy and early childhood. (After Greenwood.)

Period.	1730–49.	1750–69.	1770–89.	1790–1809.	1810–29.
Percentage deaths under 5 years	74.5	63.0	51.5	41.3	31.8

But after such a decline as these figures indicate, to continue the reduction presents a difficult problem to the administrative official. The easy part of the conflict has happened and is in the past. To continue the good fight with the same relative measure of success, one presently must needs know more precisely than is now known the pattern of the causal nexus which controls and determines the rate of infant mortality. The problem confronts the administrative official or the altruistic organization in a specific rather than a general manner. City A has a death rate under one year of age so low that even the most sanguine of hygienic optimists would hardly undertake seriously to reduce it further by any significant amount. In City B, on the other hand, babies die like flies, only somewhat more rapidly. City B differs in many respects from A. Some of these respects are such as to be easily within the power of control of a health official. Others, such as climate or the racial composition of the population, for example, are obviously beyond the possibility of any control or modification. Others lie between the two extremes, and offer practical difficulties of varying degrees. What one needs to know is which particular line of effort will in practice yield the largest return. And it is *real* knowledge not *a priori* logic that is wanted. Let a single example illustrate. It has been maintained that excessive infant mortality is primarily the resultant of the so-called ''degrading influence'' of poverty, and such a contention stirs a warmly sentimental feeling of agreement in the minds of a well-meaning public, zealous to do good. This relationship obviously *ought* to be true, therefore to a too-common type of mind it must be and is true. But Greenwood and Brown (1912) in what may fairly be regarded the most thoroughly sound, critical and penetrating contribution which has yet been made to the problem of infant mortality, are unable ''to demonstrate any unambiguous association between poverty . . . and the death rate of infants.''

The plain fact is that before control or ameliorative measures can be applied with the maximum of efficient economy to the general public health problem of infant mortality we must know a great deal more than we now do about the quantitative influence of the general factors which induce spatial and temporal differences in the rate of that mortality. But first we must get an adequate conception of the magnitude and character of the differences themselves. Let us, therefore, turn to the examination of the facts regarding variation in infant morality in the United States Birth Registration Area.

VARIATION DATA.

The material used in the following discussion has been extracted chiefly from the four annual reports on Birth Statistics issued to date. In Table 2 is given the frequency distribution showing variation in the rate of infant mortality (deaths per thousand living births) for the following groups:

1. Total population in cities of population of 25,000 or over in 1910.
2. Total population in cities of under 25,000 population in 1910.
3. Total population in rural counties of registration states.
4. White population in cities of population of 25,000 or over in 1910.
5. White population in cities of under 25,000 population.
6. White population in rural counties of registration states.
7. Colored population in cities of population of 25,000 or over in 1910.
8. Colored population in cities of under 25,000 population.
9. Colored population in rural counties of registration states.

The entries in the table are frequencies, and are to be read in accordance with the following example. There were in 1915, 29 cities of 25,000 population or more (in 1910), in the Birth Registration Area, having an infant mortality rate falling somewhere between 100 and 119 deaths under one year of age per thousand living births. All other entries are to be read in the same manner.

In order to make possible a better appreciation of the nature of the frequency distributions exhibited in Table 2, Figs. 1 and 2 have been prepared. These show for the year 1918 the frequency polygons for the total population of (*a*) cities of 25,000 and over, (*b*) cities of under 25,000, and (*c*) rural counties, and also the distributions for the white and colored population of rural counties in 1918.
and space need not be taken for the graphic presentation of the others. From Table 2 and Figs. 1 and 2 the following points are to be noted:

1. The most striking immediately observable feature of these distributions is the great range of variation which they exhibit. For example, in 1918 the 236 cities of under 25,000 inhabitants showed infant mortality rates ranging all the way from the class 40–59 deaths per thousand births to the class 300–319 deaths per thousand births. The range of variation is even greater than this in the case of the distributions for the colored population. Thus the 27 cities of 25,000 and over for which the population was divided in respect of color in 1918 show a range of variation of infant mortality from the class

TABLE 2.

Frequency distribution showing variation in infant mortality in Birth Registration Area of the United States.

Deaths per 1000 births in specified years.	Total population cities of 25,000 and over.*				Total population cities of under 25,000.*				Total population rural counties.				White population cities of 25,000 and over.*		White population cities under 25,000.*		White population rural counties.		Colored population cities of 25,000 and over.*		Colored population cities under 25,000.*		Colored population rural counties.	
	1915	1916	1917	1918	1915	1916	1917	1918	1915	1916	1917	1918	1917	1918	1917	1918	1917	1918	1917	1918	1917	1918	1917	1918
0– 19	—	—	—	—	1	1	—	—	1	—	3	1	—	—	—	—	—	—	—	—	—	—	—	1
20– 39	—	1	—	—	—	2	1	—	2	9	33	32	—	—	—	—	1	9	—	—	—	—	2	1
40– 59	2	1	5	1	11	4	12	6	49	45	152	174	1	—	—	1	30	33	—	—	—	1	8	5
60– 79	16	18	22	17	25	27	49	37	130	125	396	342	4	1	6	3	71	63	—	1	1	—	26	22
80– 99	27	24	50	43	44	42	76	65	99	107	316	298	14	13	8	5	66	64	—	1	1	1	29	19
100–119	29	34	45	40	35	39	61	48	52	57	140	165	7	8	8	6	46	38	—	1	3	1	36	42
120–139	13	14	13	27	20	23	24	38	17	21	59	64	1	5	3	4	13	16	3	1	—	4	40	37
140–159	9	5	7	13	11	7	13	15	6	15	18	31	—	—	1	5	3	8	1	2	3	1	28	30
160–179	1	2	1	1	3	5	5	15	1	2	4	11	—	—	—	2	1	2	6	2	1	2	21	19
180–199	1	—	1	2	3	6	4	4	—	—	4	8	—	—	—	—	1	—	5	2	—	—	18	17
200–219	—	—	—	—	—	—	—	6	1	—	1	1	—	—	—	—	—	1	6	5	2	2	5	10
220–239	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	3	6	5	4	8	12
240–259	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2	2	3	1	4	8
260–279	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	1	4
280–299	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	4	2	2
300–319	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	1	2	—	1
320–339	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	—
340–359	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—
360–379	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
380–399	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
400–419	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
420–439	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—
440–459	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
460–479	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
480–499	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
500–519	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—
520–539	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
540–559	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
560–579	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
580–599	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
600–619	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
	98	99	144	144	153	156	236	236	358	381	1127	1127	27	27	26	26	232	234	27	27	26	26	232	234

* In 1910.

These diagrams are fairly representative of all the distributions 60–79 deaths under one per thousand births, to the class 500–519 deaths under one per thousand births. The most extreme range is shown by the distribution for the colored population of rural counties in 1917, where the distribution extends from class 20–39 deaths under one per thousand births to the class 600–619 deaths under one per thousand births. These extraordinarily large ranges of variation demonstrate perhaps more clearly than could be done in any other way the opportunity which exists for effective administrative control and reduction of infant mortality. If there are communities, as there are in plenty, showing infant mortality rates under 100 deaths per thousand births, it suggests at once that it is possible, if the right measures are systematically and effectively applied, to reduce the infant mortality in those other communities showing very high rates to something like the level of these at present more fortunate communities.

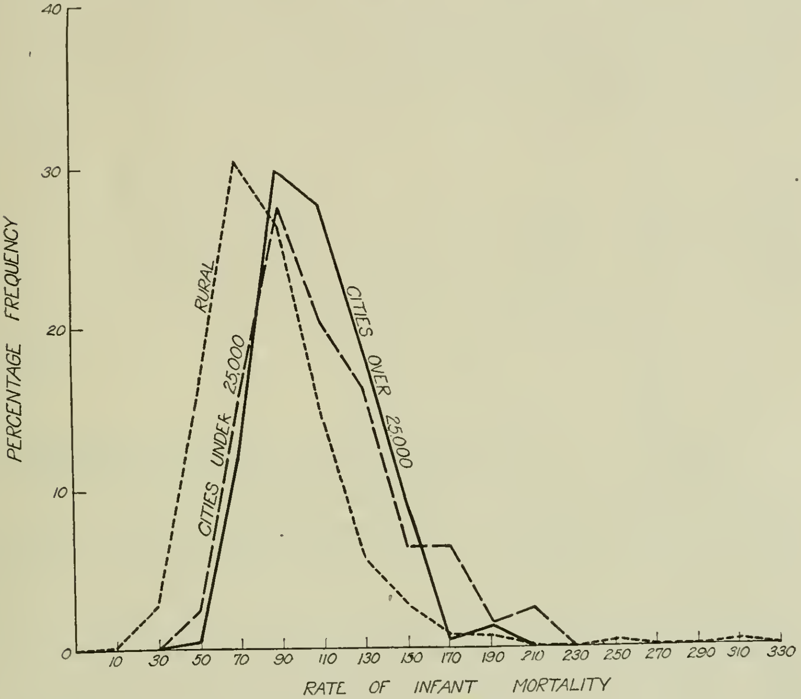

FIG. 1. Frequency polygons showing variation in the rate of infant mortality in 1918 for the total population of (*a*) cities of 25,000 and over, (*b*) cities of under 25,000, and (*c*) rural counties.

2. The distributions are all plainly of the ordinary unimodal "cocked-hat" type. There is no evidence anywhere, even in the small distributions involving only relatively few communities, of a tendency towards more than a single mode.*

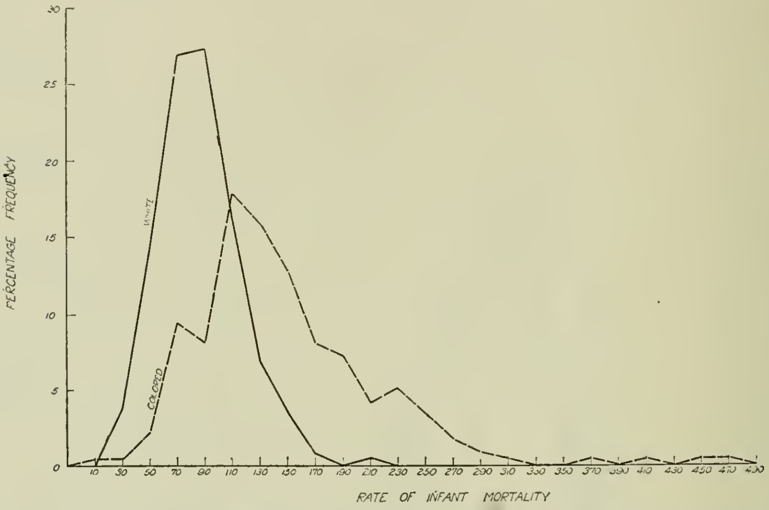

FIG. 2. Frequency polygons showing variations in infant mortality rate in 1918 of (*a*) the white population, and (*b*) the colored population of rural counties.

3. These distributions impress anyone who is at all familiar with variation curves as being unusually peaked in the region about the mode. In other words, instead of presenting a rather flat top in the modal region the tops of these curves tend rather to a sharp peak. We shall see later when discussing the analytical constants of these curves that this peaked characteristic evident on inspection is borne out by the mathematical analysis.

In Table 3 are presented the chief physical constants† of the distributions of Table 2. These constants have been determined by the method of moments from the original raw data.‡ Owing to the considerable grouping used in making up these distributions, and also because they all exhibit clear evidence of high contact at both ends of the range, Sheppard's corrections for the moments have been used throughout. In the case of the distributions divided in respect of color of the population it has not been thought advisable to calculate the higher analytical constants except in the case of the 1917 and 1918 rural distributions. In the other cases the numbers involved are too small to make the analytical treatment of any particular meaning or value.

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* For the benefit of readers not entirely familiar with modern statistical calculus it may be said that the mode of a frequency curve is the point where the maximum ordinate of the curve falls. It represents the "modal" or "fashionable" occurrence. Analytically, it is the point on the curve where $dy/dx = 0$.

† For a very brief and summarized introduction to the modern mathematical treatment of frequency curves see Pearson, K. (1914), pp. lx to lxx. References to the basic literature on the subject will be found there.

‡ I am greatly indebted to my assistants, Mr. John Rice Miner and Mrs. Charmian Howell, for aid in the arithmetical work of this paper.

The constants tabled are:

1. The arithmetic mean.
2. The median. This measures the value above and below which exactly half of the variates occur.
3. The mode. This is calculated from the relation

$$\text{Mode} = \text{Mean} - d,$$

where,

$$d = \sigma \times sk.$$

Its significance has already been pointed out.

4. The standard deviation denoted by σ. This constant measures in absolute units the degree of "scatter" or variation exhibited by the distribution.
5. The coefficient of variation. Theoretically this constant, which is the percentage of the standard deviation in the mean, has no place in discussions of variation of rates or other forms of percentages. With this fact clearly in mind I have, however, decided to table for these distributions the percentage which the standard deviation is of the mean, chiefly because of the fact that the mean rates exhibited by these distributions differ widely, particularly in the case of white and colored populations, and it seems useful to relate the variation to the mean in these cases.
6. The skewness. This constant measures the degree of asymmetry of a frequency distribution. If a distribution is perfectly symmetrical on both sides of the mean so that if folded over upon the mean as an axis the two limbs would exactly coincide, the value of the skewness is zero. It has been calculated in the present instance from the general formula,

$$sk = \frac{\sqrt{\beta_1}(\beta_2 + 3)}{2(5\beta_2 - 6\beta_1 - 9)}$$

TABLE 3.

Constants of variation in rate of infant mortality (deaths under 1 per 1000 births) computed from data of Table 1.

Group.	Mean.†	Mode.†	Median.†	Standard† deviation.	Coefficient of variation (per cent.).	Skewness.	Kurtosis.
Cities over 25,000,* Total, 1915	104.49 ± 1.78	96.26	102.76	26.14 ± 1.26	25.02 ± 1.28	+.3148 ± .0937	.145 ± .491
" " " " 1916	102.53 ± 1.67	104.47	103.24	24.69 ± 1.18	24.09 ± 1.22	−.0786 ± .0848	.224 ± .511
" " " " 1917	99.58 ± 1.32	93.83	98.00	23.45 ± .93	23.55 ± .99	+.2455 ± .0858	1.127 ± 1.558
" " " " 1918	107.78 ± 1.41	99.66	105.50	25.07 ± 1.00	23.26 ± .96	+.3237 ± .0800	.271 ± .471
Cities under 25,000,* Total, 1915	100.98 ± 1.68	92.87	97.95	30.81 ± 1.18	30.51 ± 1.28	+.1934 ± .0657	.419 ± .603
" " " " 1916	104.23 ± 1.75	97.05	101.03	32.38 ± 1.24	31.07 ± 1.30	+.2217 ± .0678	.725 ± .916
" " " " 1917	99.24 ± 1.32	84.74	94.74	29.94 ± .93	30.17 ± 1.02	+.4840 ± .1197	1.624 ± 1.197
" " " " 1918	111.61 ± 1.66	90.36	104.17	37.78 ± 1.17	33.85 ± 1.13	+.5625 ± .2647	3.212 ± 2.945
Rural counties, Total, 1915	83.07 ± .85	75.40	79.54	23.95 ± .60	28.83 ± .79	+.3204 ± .1454	2.200 ± 2.690
" " " 1916	85.28 ± .90	76.10	82.15	29.94 ± .63	30.42 ± .81	+.3536 ± .0509	.362 ± .319
" " " 1917	82.01 ± .52	74.73	78.96	25.71 ± .37	31.35 ± .49	+.2833 ± .1157	2.392 ± 2.379
" " " 1918	84.43 ± .57	72.14	80.97	28.40 ± .40	33.64 ± .48	+.4328 ± .0409	1.281 ± .482
Cities over 25,000,* White, 1917	92.22 ± 2.02	—	92.14	15.60 ± 1.43	16.91 ± 1.60	—	—
" " " " 1918	102.59 ± 2.00	—	99.23	15.42 ± 1.42	15.03 ± 1.41	—	—
Cities under 25,000,* White, 1917	98.46 ± 2.75	—	97.50	20.82 ± 1.95	21.15 ± 2.06	—	—
" " " " 1918	114.62 ± 4.17	—	113.33	31.49 ± 2.95	27.47 ± 2.80	—	—
Rural counties, White, 1917	86.21 ± 1.07	81.66	84.24	24.15 ± .76	28.02 ± .99	+.1799	2.847
" " " 1918	85.90 ± 1.27	77.80	83.75	28.90 ± .90	33.65 ± 1.05	+.2802 ± .0650	.999 ± 1.055
Cities over 25,000,* Colored, 1917	202.59 ± 8.88	—	194.00	68.43 ± 6.28	33.78 ± 3.44	—	—
" " " " 1918	216.67 ± 11.15	—	214.00	75.87 ± 7.88	39.63 ± 3.65	—	—
Cities under 25,000,* Colored, 1917	213.08 ± 9.92	—	228.00	74.96 ± 7.01	35.18 ± 3.68	—	—
" " " " 1918	217.69 ± 11.46	—	225.00	86.65 ± 8.10	39.80 ± 4.27	—	—
Rural counties, Colored, 1917	134.76 ± 2.55	106.17	127.25	57.37 ± 1.80	42.57 ± 1.56	+.4984 ± .5222	4.036 ± 6.160
" " " 1918	147.26 ± 2.92	108.83	134.59	66.15 ± 2.06	44.92 ± 1.66	+.5819 ± .4154	4.239 ± 4.998

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* In 1910.

† In concrete units, i.e., rate of deaths under 1 per 1000 births.

7. The kurtosis. This constant which in terms of the moments has the following value

$$\eta = \beta_2 - 3$$

measures the extent to which a curve in the region about the mode is more or less flat-topped than the normal or Gaussian curve. The introduction of this constant we owe to Pearson (1905). Curves on which the kurtosis has a negative value ($\beta_2 < 3$) are more flat-topped than the normal curve and are called platykurtic, while those which are more peaked than the normal curve have a positive value of the kurtosis ($\beta_2 > 3$) and are called leptokurtic.

From the data presented in Table 3 the following points are to be noted:

1. There is no certainly significant decline in the mean value of the rate of infant mortality during the four years covered by these statistics in any of the demographic units considered. In the cities of over 25,000 the mean rate declined during the years 1915, 1916, and 1917, but the total amount of this drop cannot be regarded as statistically significant, having regard to the probable errors involved. In other words, the change from a mean rate of 104 in 1915 to a mean rate of 100 (99.58) in 1917 may easily have been simply the result of chance.

2. In 1918 there was a general tendency towards an increase in the mean rate of mortality over that which obtained in 1917. This increase is unquestionably to be attributed to the influenza epidemic of the autumn and winter of 1918. A careful examination of the rates by months will convince one that the mortality of infants increased very materially during the period of the epidemic. Whether this increased number of deaths was truly to be charged to influenza does not concern us here. The important fact is that the rate of infant mortality markedly increased coincidently with the existence of the epidemic. In a number of cases the increase in the mean rate of 1918 over 1917 is probably statistically significant having regard to the probable errors involved. Thus we have the following large differences:

Cities under 25,000, Total, 1918 mean to 1917 mean $= 12.37 \pm 2.12$
Cities over 25,000, White, 1918 mean to 1917 mean $= 10.37 \pm 2.84$
Cities under 25,000, White, 1918 mean to 1917 mean $= 16.16 \pm 5.00$

It is noteworthy that this increase in the infant mortality rate in 1918 is practically confined entirely to the cities. The rural counties, whether for white or colored or total population, show little or no change in 1918 as compared with 1917.

3. There is no unequivocal difference in the mean rates of infant mortality in the larger as compared with the smaller cities. Considering the largest differences in mean rates for total populations in cities of 25,000 and over, as compared with cities of under 25,000, there is no difference which is as much as even three times its probable error. This result that there is no marked or striking difference in the mean rate of infant mortality in large as compared with small cities is somewhat surprising. It suggests by inference that when the matter is adequately investigated it will probably be found that there is no definite or significant correlation between the rate of infant mortality and the density of population in American cities. It should be understood, however, that this is here suggested only as a probable inference. Positive statements on the matter cannot be made until the point has been carefully investigated by the method of multiple correlation. The suggestion which these figures make, however, is in accord with the results of the writer's studies (Pearl, 1919 and 1921) on the correlation between the influenza mortality and density of population, where it is shown that there is no sensible net correlation between these two variables.

4. The mean rates of infant mortality are notably smaller in the rural than in the urban areas. This fact has, of course, long been well known. The first writer on vital statistics, in the sense in which we now understand that subject, Captain John Graunt (1662), more than 250 years ago pointed out that rural communities exhibited generally a lower rate of mortality than urban communities. We are still nearly as far as he was from a scientific understanding of *why* this is so. There has long been current a certain glib patter of explanation for the superiority of rural communities over urban in rate of mortality, but the subject still awaits careful analytical quantitative investigation, which will measure the relative influence of each one of the considerable number of factors which may be obviously directly concerned in producing this difference. The difference between urban and rural rates of infant mortality is reflected just as clearly in the high absolute rates of the colored population as it is in the lower rates of the white population.

5. The mean rates of infant mortality are, roughly speaking, some-

thing like twice as high for the colored population as for the white population in each of the demographic units considered, and at all times. This, again, is a fact in general well known, but here we have precise figures on the point, with probable errors, which show definitely how tremendously poorer the negro baby's chances of surviving the first year of life are than the white baby's.

6. The mode is seen in every case but one (1916, cities over 25,000, total) to be smaller than the mean; that is, to lie to the left of the mean in the distribution. The differences between mean and mode are fairly considerable for most of the distributions. They are largest in the case of the rural colored distributions.

7. The cities of over 25,000 exhibit distinctly less variation in respect of infant mortality than do either the smaller cities (under 25,000) or the rural counties. This is true, however the variation is measured, whether absolutely, in terms of standard deviation, or relatively in percentage terms. The smaller cities and the rural counties exhibit about the same degree of variation relative to their means, but absolutely, in terms of standard deviation, the rural counties show less variability than the cities under 25,000. It is probable that in this case the coefficient of variation represents more truly the real biological fact than the standard deviation. The colored distributions exhibit a much higher degree of variation in respect of infant mortality, however measured, whether absolute or relative, than do the white populations. Probably part of this greater variation in the colored population arises from the fact that these populations are absolutely much smaller in size in every case than the white populations of the same communities, and therefore less likely to give steady and characteristic rates. How much of the actually observed variation, however, is to be explained in this way is at present undeterminable. In general, it may fairly be assumed that the greater the variation exhibited by a given class of the community in respect of infant mortality, the greater the chance of effective control and reduction of the average infant mortality by administrative measures. There can be no question that there is no field which offers so great opportunities in this direction as the colored population.

8. The skewness is seen to be positive in sign in every case but one. In that case (1916, cities over 25,000, total) the skewness is not significant in comparison with its probable error. With this exception the curves tend to tail off more gradually and farther toward the right end than toward the left end of the range, and in consequence,

as we have already seen, the mode lies to the left of the mean. In many instances (notably in the distributions for the colored population of rural counties) the skewness values rise to considerable magnitudes and may be regarded as significantly different from zero, having regard to their probable errors. In other words, the rate of infant mortality in these different American demographic units tends generally to distribute itself in a substantially unsymmetrical fashion about the mean, extremely high rates occurring more frequently than correspondingly low rates. This fact might perhaps be taken to indicate that the task confronting the administrative control of infant mortality in certain communities of the United States and yet to be accomplished is even greater than what has already been accomplished in the past, great and worthy of commendation as that is.

9. The kurtosis is seen to be positive in sign and relatively large in amount in most cases. This confirms analytically the conclusion already reached from mere inspection, namely, that the curves of variation in infant mortality are, with great uniformity, leptokurtic; that is, more sharply peaked than the corresponding normal curve would be.

10. A noteworthy point is the remarkable similarity, evident both from inspection and from the analytical constants, in all of these frequency distributions. Evidently infant mortality variation curves are of a quite definitely characteristic and uniform type, at least in this country. It will be interesting to examine similar curves for other countries.

For the sake of further analytical work the moments and certain derived constants from the longer distributions are given in Table 4.

It is of some interest to compare the variation constants here exhibited with those for other countries. Greenwood and Brown (1912, p. 43) give means and standard deviations for infant mortality rates for Bavarian communities computed from the data of Groth and Hahn (1910). Taking their longest series (161 observations) and changing from rates per 100 living births, as they state the figures, to rates per 1,000 births to correspond with other data in this paper, we have:

Mean infant mortality rate (1900–1904) .... = 243.09
Standard deviation .... = 76.42

It is at once evident that these figures are of about the same order of magnitude as those shown in Table 3 for the colored population of cities in the United States Birth Registration Area. The percentage

TABLE 4.

Analytical constants for infant mortality curves.

Group.	N	ν_1†	μ_2†	μ_3†	μ_4†	β_1	$\sqrt{\beta_1}$	β_2
Cities over 25,000,* Total, 1915	98	2.7245	1.7081	1.1649	9.1772	.2723	.5218	3.1454
“ “ “ “ 1916	99	3.6263	1.5245	.3296	7.4924	.0307	.1751	3.2440
“ “ “ “ 1917	144	2.4792	1.3746	1.0234	7.7984	.4033	.6350	4.1274
“ “ “ “ 1918	144	2.8889	1.5710	1.1084	8.0719	.3168	.5629	3.2706
Cities under 25,000,* Total, 1915	153	4.5490	2.2734	1.5528	19.2617	.1804	.4247	3.4194
“ “ “ “ 1916	156	4.7115	2.6219	2.2304	25.6044	.2760	.5254	3.7246
“ “ “ “ 1917	236	3.4619	2.2415	3.3963	23.2314	1.0243	1.0121	4.6218
“ “ “ “ 1918	236	3.0805	3.5670	9.2407	79.0312	1.8302	1.3529	6.2116
Rural counties, Total, 1915	358	3.6536	1.4336	1.5898	10.6856	.8579	.9262	5.1995
“ “ “ 1916	381	2.7638	1.6824	1.3432	9.5167	.3789	.6155	3.3623
“ “ “ 1917	1127	3.6007	1.6525	1.8928	14.7237	.7939	.8910	5.3916
“ “ “ 1918	1127	3.7214	2.0165	2.4618	17.4078	.7391	.8597	4.2810
Rural counties, White, 1917	232	2.8103	1.4583	1.2374	9.2685	.4937	.7027	5.8472
“ “ “ 1918	234	2.7949	2.0883	2.0117	17.4371	.4444	.6666	3.9986
Rural counties, Colored, 1917	231‡	5.2381	8.2279	33.3269	476.3210	1.9940	1.4121	7.0359
“ “ “ 1918	234	6.8632	10.9407	54.8500	866.4468	2.2973	1.5157	7.2386

* In 1910.

† It should be expressly noted that the values of the moments ν_1 (about arbitrary origin at the lower range end), and μ_2, μ_3 and μ_4 (about the mean) as here given, are in *units of grouping*, and not in terms of death rates. The unit of grouping, as can be seen in Table 1, is 20 deaths per 1000 births.

‡ These are the constants after dropping the single aberrant observation in the death rate class 600–619 deaths per 1000 births.

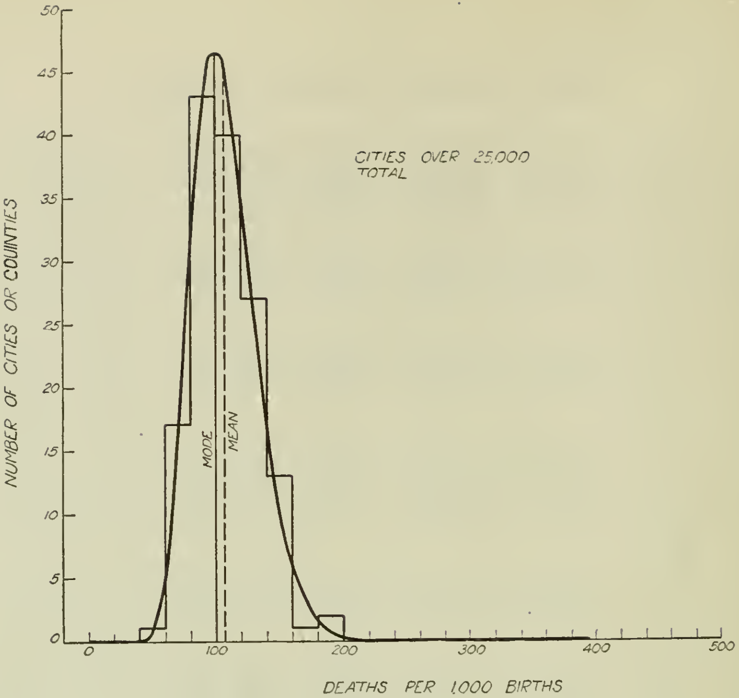

FIG. 3. Frequency histogram and fitted skew curve for variation in the total rate of infant mortality in 1918 in cities of over 25,000 population (in 1910).

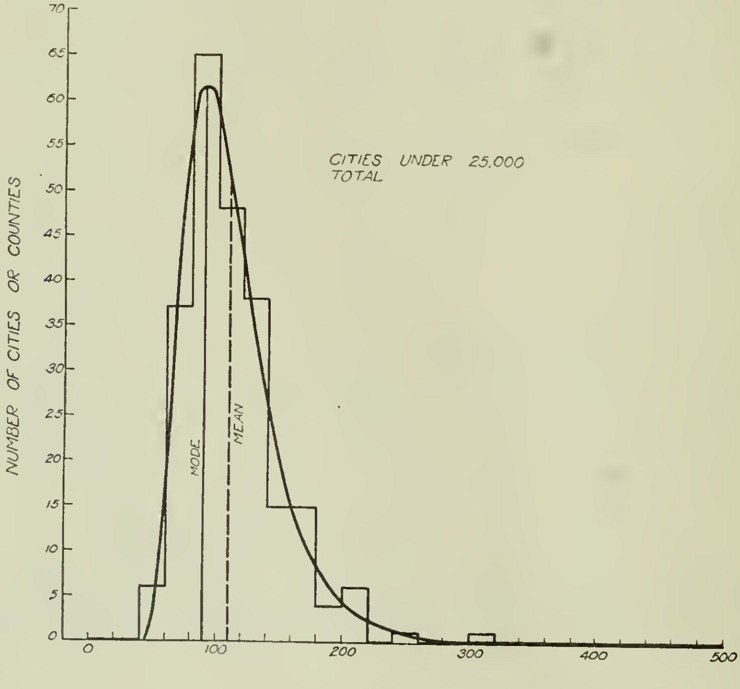

FIG. 4. Frequency histogram and fitted skew curve for variation in the total rate of infant mortality in 1918 in cities of under 25,000 population (in 1910).

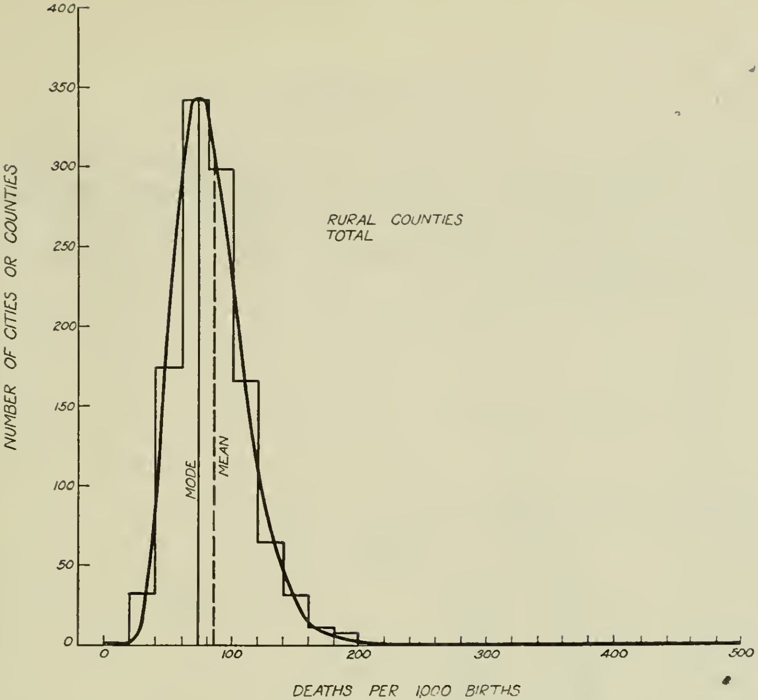

FIG. 5. Frequency histogram and fitted skew curve for variation in the total rate of infant mortality in 1918 in rural counties of the Birth Registration Area.

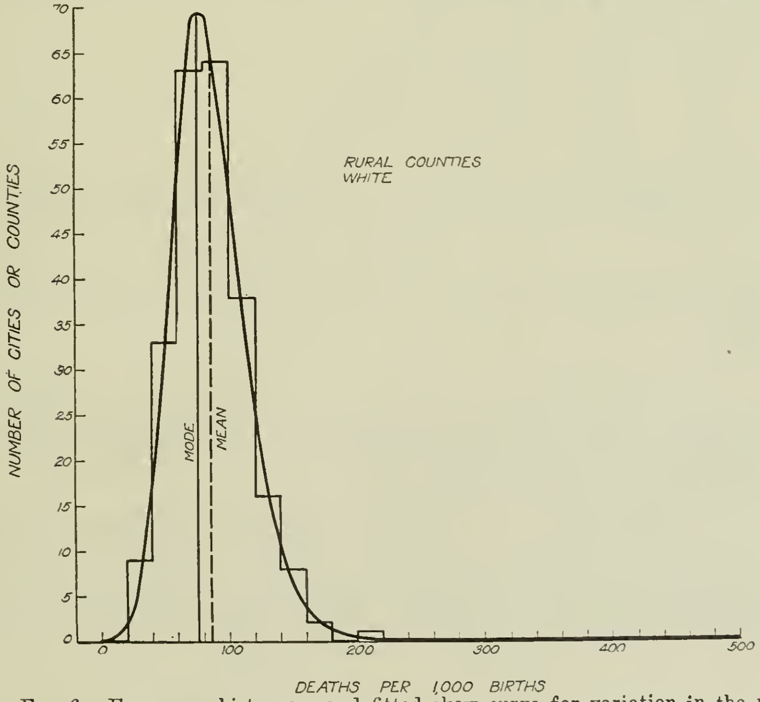

FIG. 6. Frequency histogram and fitted skew curve for variation in the rate of infant mortality among whites in 1918 in rural counties of the Birth Registration Area.

of the standard deviation in the mean for the Bavarian data is 31.44 per cent., indicating that relatively as well as absolutely the degree of variation was about the same in the infant mortality of these Bavarian communities twenty years ago as in our urban colored populations at the present time.

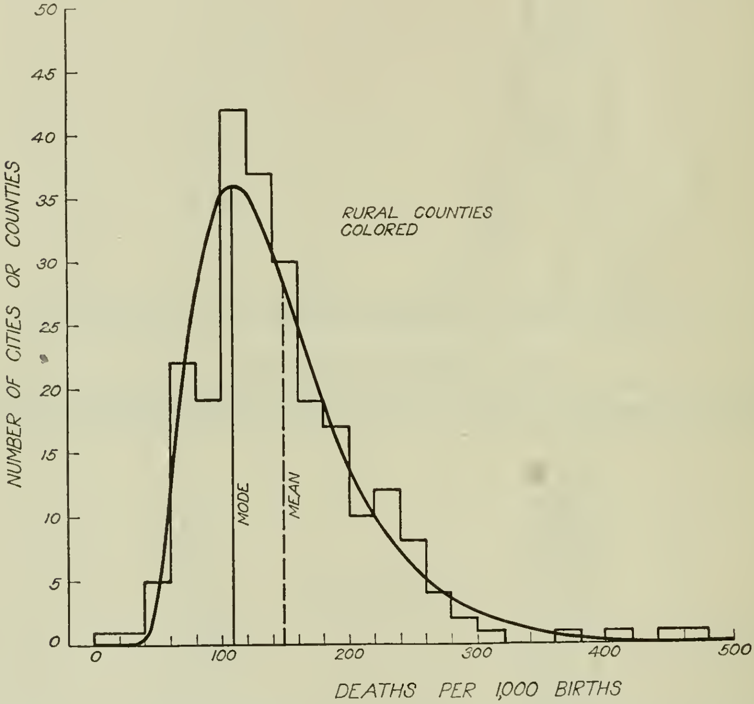

FIG. 7. Frequency histogram and fitted skew curve for variation in the rate of infant mortality among negroes in 1918 in rural counties of the Birth Registration Area.

It has seemed desirable, in the case of certain of the distributions, which may be fairly considered as typical representatives of all, to go on and fit the appropriate skew frequency curves to the observations. The results are shown in Figs. 3–7.

It is evident that the curves in general fit closely the observed facts. The greatest discrepancy between theory and observation is found in Figure 7, the negro rates for rural counties. Here the observations are obviously rough, and probably the curve given is as satisfactory a result as could be obtained with such material.

The equations for the curves are as follows:

Cities over 25,000, total:

$$\text{(Type I)} \quad y = 46.5502 \left(1 + \frac{x}{2.8946}\right)^{4.4296} \left(1 - \frac{x}{14.5155}\right)^{22.2126}$$

Cities under 25,000, total:

$$\text{(Type VI)} \quad y = 8.8238 \times 10^{35} \, (x - 18.4653)^{2.9924} \, x^{-26.7505}$$

Rural counties, total:

$$\text{(Type VI)} \quad y = 9.2560 \times 10^{70} \, (x - 17.7766)^{8.9630} \, x^{-55.2026}$$

Rural counties, white:

$$\text{(Type IV)} \quad y = 0.005609 \left[1 + \frac{x^2}{(4.5095)^2}\right]^{-12.5407} e^{25.9862} \tan^{-1} \frac{x}{4.5095}$$

Rural counties, colored:

$$\text{(Type VI)} \quad y = 4.2887 \times 10^{27} \, (x - 20.9629)^{3.0627} \, x^{-19.9804}$$

CONCLUSIONS.

The facts which are set forth in this paper, being wholly objective, and based upon data which are in the aggregate highly accurate—being simply registered births as a whole and registered deaths under one year of age as a whole, both registrations being subject at every step to close control and scrutiny by both local and federal officials—lead to certain definite and clean-cut conclusions, to wit:

1. Infant mortality rates exhibit a relatively high degree of place variation in both rural and urban areas of the United States. This high variation is evident upon any and every basis of estimation which may be chosen, whether range, standard deviation, or coefficient of variation.

2. This great variation demonstrates the opportunity which still exists for effective administrative control and reduction of infant mortality.

3. The distributions of place variation in infant mortality are unimodal for both urban and rural, white and colored populations.

4. There was no certainly significant decline in the mean or average rate of infant mortality in the United States Birth Registration Area during the years 1915–18, inclusive.

5. Presumably as a result of the influenza epidemic the mean infant mortality rates were higher (in some cases significantly so) in 1918 than in 1917 in cities. No such difference is exhibited by the mean rates for rural areas.

6. There is no significant difference in the mean infant mortality rates in large cities (over 25,000 population) as compared with small cities (under 25,000 population) in the United States Birth Registration Area.

7. The mean rates of infant mortality are notably smaller in the rural than in the urban areas, in both the white and the negro elements of the population.

8. The mean rates of infant mortality are something like twice as high for the colored population as for the white population in each of the demographic units considered (large cities, small cities, rural areas), and at all times.

9. Cities of over 25,000 population in the Birth Registration Area exhibit distinctly less variation in respect of rate of infant mortality than do either the smaller cities or the rural counties.

10. The colored population exhibits a much higher degree of variation, however measured, in respect of infant mortality than does the white population.

11. The place variation in infant mortality rates is generally significantly unsymmetrical or skew in the positive deviation.

12. The kurtosis is positive in sign and relatively large in amount in most cases.

13. The observed distributions of variation in infant mortality rates in the United States Birth Registration Area are satisfactorily graduated by Types I, IV and VI of Pearson's skew frequency curves.

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. GIARDIA ENTERICA: A PARASITIC INTESTINAL FLAGELLATE OF MAN.*

By CHARLES E. SIMON, B.A., M.D.

Plates XXII to XXIV.

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Introduction.

In one of his early papers on human giardiasis Grassi (1881) expressed the opinion that the infestation of man was in all probability brought about by the ingestion of food contaminated with the feces of correspondingly infested rodents. He was led to this conclusion by the discovery of organisms in the common house mouse (*Mus musculus*), the black rat (*Mus rattus*), the Norwegian rat (*Mus decumanus*), the meadow mouse (*Arvicola arvalis*) and the wood mouse (*Arvicola silvestris*), which he evidently regarded as identical with those occurring in the human being. He also found giardias in cats, dogs, sheep and rabbits, but apparently did not regard these as important agents in the production of the human infestation. He mentions particularly that cats are rarely infested, but makes no statement regarding the frequency of the occurrence of the parasite in dogs, sheep and rabbits. His belief that the rodents in question are responsible for the human infestation is based upon the frequency of the infestation of these animals and the common custom among the peasants of northern Italy of keeping their bread in open granaries, where rats and mice have ready access to it and can contaminate it with their feces. He mentions that he has frequently noted this himself.

The doctrine that the human infestation is probably of rodent origin has been handed down in the text-books, ever since, notwithstanding the fact that no proof of the identity of the forms found in man and those occurring in animals, nor indeed of the animal forms amongst each other, has ever been adduced. The entire problem of giardiasis in the human being has, indeed, received but scant attention in the past, and up to the time of the great war the majority of the papers dealing with the subject were essentially devoted to the recital of isolated cases or small groups of cases. The infestation was largely looked upon as a medical curiosity and as of negligible importance. Very little was known of the geographical distribution of the parasite, of the extent of its occurrence in those countries where it had been observed, of its pathogenicity, of the mode of infection and hence of its control. Up to the present time (1921) an adequate morphological basis for comparison of the human type with the forms found in various animals did not even exist. A considerable amount of interest in these various questions was, however, evoked by experiences gained in the course of the war. It was then found that the infestation of man was much more common than was formerly believed, that many individuals harbor the parasite without evidence of any harmful effects, it is true, but that in many others obstinate diarrhoea exists which can not be attributed to any other cause. But even so, we are evidently only beginning to appreciate that giardia is a parasite which is worthy of study. During the past two years the writer has devoted a considerable part of his time to the investigation of some of these problems, and it is intended to present the results of his investigations in a series of papers, of which this is the first.

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The present paper is the first of a series which the writer is about to present on the general subject of Giardiasis.

The problem.

The present paper is intended as a contribution to the question whether human giardiasis is of rodent origin. To determine this point it is essential to develop a picture of the parasite, as it occurs in man, which may serve for comparison with the forms which occur in the animals under consideration. Up to the present time such a standard has not been available. There has been a gradual evolution of the final picture, as will appear from a survey of the work done in the past, but even the latest account, given in 1912, leaves many points undetermined. Before proceeding to this survey a few words regarding the taxonomics of the organism may not be out of place.

Taxonomics.

The organism which was discovered by Lambl in 1859 was thought by this observer to belong to the genus Cercomonas. From its habitat he termed it *Cercomonas intestinalis*. Grassi (1879), who apparently was in ignorance of Lambl's discovery, described what was evidently an organism belonging to the same genus, but occurring in various rodents, under the name *Dimorphus muris.* He expressed the opinion that this organism was identical with one which he had encountered a short while before in the human being. Two years later (1881) he abandoned the term *Dimorphus muris* and named the parasite *Megastoma entericum.* Bütschli (1886) in reviewing the taxonomic status of the organism subsequently changed this to *Megastoma intestinalis,* while Blanchard (1888) suggested the name *Lamblia intestinalis* to commemorate the name of its actual discoverer. Stiles (1902) still later changed Blanchard's species name to *duodenalis.* Kofoid (1915) finally proposed the name *Giardia lamblia* Stiles in *litt.* for the same form, and still more recently (1920) changed this to *Giardia enterica,* which, according to the rules of nomenclature, would be correct, providing that the mammalian genus Giardia is, indeed, identical with that originally described in tadpoles, and to which Künstler in 1882 and 1883 first applied the name *Giardia.**

HISTORICAL SURVEY OF THE EVOLUTION OF OUR KNOWLEDGE OF THE MORPHOLOGY OF GIARDIA ENTERICA.

(*a*) *The trophozoite.*

It should be stated in advance that up to the year 1908, when Bensen first questioned the identity of the human and the animal forms of Giardia, those studies which dealt with the morphology of the organism in greater detail, like that of Grassi and Schewiakoff (1888), Metzner (1901) and Wenyon (1907), had reference to animal forms exclusively, and the writers evidently assumed that these were identical with the human form.

Our knowledge of the structure of the human type, up to the year 1908, was based entirely upon relatively inadequate studies of the organism on the part of various clinicians. From these it was apparent that *Giardia enterica* was without a doubt closely related to the organisms described by Grassi and Schewiakoff, Metzner and Wenyon. No detailed description based on more modern methods of investigation was given, however, until 1908, in which year Bohne and v. Prowazek published the first account of the structure of the human form that is comparable with the description which Metzner gave of the parasite found in rabbits and Wenyon of that encountered in culture mice. Barring Grassi and Schewiakoff's study, which can only be viewed as a retrogression from Grassi's original description of Giardia, and for which Schewiakoff is evidently solely responsible, the study of the animal forms had progressed far beyond our knowledge of the human type, when Bohne and v. Prowazek's and Bensen's papers appeared. In 1909 followed Noc's account and in 1912 that of Rodenwaldt. The minute studies of Kofoid and Christiansen (1915) and of Boeck (1919) have reference only to animal material.

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* In a recent contribution entitled *Giardia-Lamblia* by F. Reuling and E. Rodenwaldt (Arch. f. Protist., 1921, XLII, 337–345) the writers discuss the validity of suppressing the genus name *Lamblia,* as being synonymous with *Giardia.* They conclude that there is no sufficient basis for regarding the two as separate genera, but that it might be justifiable to create two subgenera, under the genus *Giardia,* viz., Subgenus *Giardia s. str.* and subgenus *Lamblia.*

Lambl's original description of the organism (1859) as he saw it in the " jelly "-like stools of children, though lacking almost entirely in detail, is sufficiently succinct as to leave no doubt that his Cercomonas and the Giardia of the present day are the same. He likened its form to that of the tadpole—rounded off in front, coming to a point behind—and pointed out that in its anterior portion there is a roundish-oval sucking cup, at the bottom of which two little structures, resembling nuclei, could be discerned. He found the length of the animal to vary between 18 and 21 μ, of which 3 to 4 μ were represented by the tail; for the breadth Lambl gives 8 to 16 μ as measurements. When dying the periphery of the sucking cup was seen to contract and to expand in a more or less rhythmical manner. In neither his first (1859) nor his second (1860) paper does he mention the presence of flagella. His illustrations will be found reproduced in Plate XXII, Figs. 1 and 2. Lambl states that he looked for cilia about the oral aperture, but found none, but that he does not wish to deny their existence altogether. In this connection it may be interesting to note a statement by Lambl (1859) to the effect that " Die jüngsten Formen gleichen ovalen gallertig aufgeschwollenen Schleimkörperchen." This suggests that he saw not only the vegetative form of the organism, but also the corresponding cysts, the discovery of which has heretofore been accredited to Perroncito.

Cunningham (1872) evidently encountered the same organism in the course of his examinations of cholera stools in India, as well as in the movements from cases of " simple diarrhoea," but did not give it a name. He merely speaks of a monad with a form " which is flattened out like a spatula, convex on one side, concave on the other, and drawn out to a delicate filiform appendix, in the neighborhood of which on the concave surface several small cilia can be discerned in process of rapid vibration." Evidently Cunningham deserves the credit for having first recognized the existence of the so-called ventral flagella, for these are the only ones to which the description "in process of rapid vibration" could apply.

In 1879 followed Grassi's description of the human form (1879*a*). In his first account he states that at the anterior end he saw flagella emerge, "four at the most," and about twice as long as the body of the animal, which measured 7 μ in length by 4 μ in breadth. The posterior end presents a thread-like prolongation almost as long as the body; evidently this description can have reference only to the caudal flagella which he then did not recognize as being paired. Near the anterior pole of the body proper Grassi saw in "some specimens evidence of a nuclear structure." In a short note recording his *Dimorphus muris,* published a little later in the same year (1879*b*), he states that 6 flagella originate on the plano-concave surface at the junction of the concave and the flat portion, and a little posteriorly in the median line. "Dalla faccia piana-concava e precisamente dal limite fra la parte piana e la concava e piu indietro, sempre sulla linea mediana originano sei flagelli."*

A more detailed description of the organism then appeared in 1881. In this he speaks of the kidney form of the sucking cup, with its long axis placed at right angles to the long axis of the body of the animal, the oval form of the two nuclei, the thickening of the chitinous envelope around the margin of the suckers, the presence of a mid-rib extending from the posterior margin of the cup to the end of the tail, as also of a small rounded nucleiform body, a little posterior to the anterior end of the rib. He further mentions that the organism has eight flagella, four of which he pictures as originating from the cytostomal border and two from the lateral margin of the body, while the remaining two are shown as starting from the tip of the tail. In this publication he gives the length as varying between 5 and 10 μ and the breadth between 4 and 6 μ. In conclusion it may be added that Grassi also mentions the presence of numerous elliptical bodies in the stools of his patients, but gives no measurements or further description. The probability is that these bodies were encysted giardias.

Grassi's conception of the structure of the organism may be seen at a glance by reference to the reproduction of his original illustrations in Plate XXII, Figs. 3 and 4.

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* From the context it is not clear whether this description had reference to the human or rodent form.

During the long interval from 1881 to 1908 no essential additions were made to our knowledge of the morphology of the human type of Giardia, while the forms occurring in rabbits and in culture mice were studied in great detail. With these studies, however, we shall not concern ourselves in the present paper, although they unquestionably must have exerted an influence upon the observers who, in 1908 and subsequently, took up a more detailed study of the human form.

The investigations of Bohne and v. Prowazek (1908) were based upon the study of material which was obtained from two individuals who had been suffering from diarrhoea, the one for three weeks, the other for four days preceding admission to the hospital. The writers themselves evidently regarded their studies as incomplete, for they state specifically that the morphology and development of the organism could not be worked out adequately because the patients required treatment. Notwithstanding, they have made material contributions to our knowledge of the structure of the organism, as will appear below. Of particular interest is their conception of the origin of the flagella (Plate XXII, Fig. 5*a* and *b*). They picture the caudal flagella as continuing forward in the form of two axostyles, into what we may call the peristomal vestibule, and extending not quite as far as an imaginary line connecting the anterior poles of the two nuclei, where they end each in a granule. From these same granules the postero-lateral flagella are pictured as taking their origin, and each ventral flagellum from a granule located posterior to the anterior terminal granules of the postero-lateral flagella and anterior to the club-shaped nucleoid body of Grassi, which the writers of those days variously speak of as the "problematische Körper" or the "räthselhafte Körper," and which Bohne and v. Prowazek recognize as extending approximately to the middle of the body in a dorso-ventral direction, without reaching the dorsal surface. Immediately in front of the terminal granules just mentioned the writers picture two additional granules which give rise to the antero-lateral flagella.

A comparison of their illustrations with those of Grassi will show the radical difference in the conception of the origin of the flagella on the part of these writers.

In the nucleus Bohne and v. Prowazek recognized the presence of a karyosome and running forward from this of a rhizoplast-like fibril which connects with a granule lying in the nuclear membrane (see Plate XXII, Fig. 5*b*). From each one of these in turn they picture a similar fibril running forward and inward and meeting its fellow in a centrally placed granule.

Bensen's description of the organism which appeared in the same year (1908) is of particular interest, in as much as he was the first, and it may be added that he has been the only writer, who has thus far attempted to establish a standard for the human form for purposes of comparison with forms occurring in the lower animals.

Bensen describes the human form as follows (see Plate XXIII, Fig. 1): It is slender, not much wider in the middle than anteriorly; posteriorly the lines run almost straight to the tail end. There are four basal granules, *lying side by side,* between the anterior poles of the nuclei; they are joined together by a fine fibril which is slightly arched. The outer pair of granules is united to the karyosome of the corresponding nucleus by a rhizoplast which first connects with the centrosome, lying in the nuclear membrane, and then proceeds to the karyosome. The antero-lateral and the postero-lateral flagella start from the outer granules, while the caudal flagella are united with the central granules by means of *two* axostyles. The ventral flagella originate from two granules which are located on the axostyles. At the points where the antero-lateral, the postero-lateral and the caudal flagella leave the body proper Bensen pictures a basal granule. The "problematic body," the parabasal body of Kofoid and his school, is described as very large, as occupying a transverse position just posterior to the points of origin of the ventral flagella, and as somewhat thicker in the center, but not becoming much thinner toward its rounded ends.

Noc's account which followed in 1909 is largely devoted to a consideration of the encysted animal. He points out, however, that in the vegetative form the third nucleus, as he terms the "mysterious body," may vary considerably in size and form; that occasionally it is no larger "than the granules from which the anterior flagella spring," while on the other hand it may be quite voluminous; that in some specimens and even in the same preparations it may present a more or less elongated oval form or appear as a "fuseau a deux branches distinctes," or even as a semilunar arc. He looks upon this body as a " reserve de chromatine " (see Plate XXIII, Fig. 2*a*).

In 1912 finally appeared Rodenwaldt's paper, which represents the most detailed description of the organism to date. It is based upon material obtained from two cases. He gives the length as varying from 10 to 25 μ and states that the breadth also is quite variable. Like Noc he does not think that different species can be established on the basis of differences in size. Young forms he says "are generally speaking longer and more slender, while old forms are broad and plump." The sucking cup he views as composed of *two* cup-like depressions which are fused together. The dorsal surface becomes arched more and more as the organism grows older, and this continues until the dorsal region gradually curves over the sides toward the flat ventral surface, which latter in extreme cases appears almost as a shield that has been pressed into a soft ball. He gained the impression that the posterior portion of the body was bordered by a marginal fibril, which is said to appear most distinctly in older organisms. He pictures four basal granules just anterior to an imaginary line uniting the anterior poles of the two nuclei (Plate XXIII, Fig. 3). Of these the central pair lie anterior to the outer pair. The anterior central pair are united with each other by a fine fibril, as also with the outer pair, which in turn are linked with the karyosomes of the respective nuclei by way of a large mass of chromatin which is located in the nuclear membrane of the anterior pole. The peristome, according to Rodenwaldt, is bounded by a stout fibril which starts from one of the basal granules just mentioned, and then surrounds the peristome to end in the corresponding outer granule of the other side. The flagellar apparatus, so far as the anterior, the lateral and the caudal flagella are concerned, takes its origin from the four granules mentioned. The two caudal flagella are connected with the two anterior and centrally located granules by two axostyles. The intra cytoplasmic portions of the two lateral flagella run to the same granules. The two anterior flagella on the other hand Rodenwaldt pictures as arising from the posterior and outer granules from which the peristomal fibril takes its origin. He adds that he has never seen a basal granule at the point of exit of either the anterior or the lateral flagella, such as Bensen claims and pictures, while caudal granules are usually quite distinct. The ventral flagella are represented as originating from two thickenings in the two axostyles in their anterior third. The " unerklärliche " body he found absent in very strong animals, while in somewhat older ones it appeared as a compact mass and in old animals in the form of two or three delicate clasps (Spangen). Rodenwaldt is the first who has given an account of the changes which occur in the nuclei preparatory to division.*

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* To judge from a somewhat fragmentary preliminary account of a forthcoming renewed description of *Giardia enterica* (given in Reuling and Rodenwaldt's paper, referred to in the foot note on p. 442) Rodenwaldt now conceives his peristomal fibril as forming a convex curve in its posterior portion, running forward and ventrally, with the axostyles and postero-lateral flagella passing

In Plate XXIII, Fig. 4, finally the writer has reproduced one of a pair of dividing organisms, pictured by Wenyon and O'Connor in their monograph on ''Human Intestinal Protozoa in the Near East'' (1917) which will give an idea of the structure of the organism as they conceived it to be, though they give no detailed description.

(*b*) *The encysted form.*

While both Lambl and Grassi probably saw the cysts of the human Giardia in their preparations, they evidently did not recognize their relation to the vegetative form. The credit for having established this for the first time belongs to Perroncito (1887). He states that he found that the vegetative forms predominated in the stools of his patients when there was diarrhoea, while cysts only were found when there was constipation. He does not give a detailed description of the cysts and evidently did not study any stained specimens. He merely states that ''the little bodies which are round or oval show distinctly cellular contents, amongst which a flagellum can be discerned.'' As measurements he gives 17 μ for the length and 10 to 12 μ for the breadth. He claims that in soft stools the organisms may be found in various stages of encystment. Regarding the significance of the process of encystment he concludes that the cysts are destined to keep the parasites alive and to bring about their dissemination.

The cysts have subsequently been mentioned by practically all observers who have come in contact with cases of giardiasis, but no attempt to study their morphology in detail was undertaken until Rodenwaldt did so in 1912.

Salomon (1899), however, noted the presence of 1 to 3 slightly curved lines and near their origin small nuclear structures in addition to certain sickle shaped markings.

v. Prowazek (1908) recognized that within the cyst the organism undergoes binary division, the resulting individuals subsequently becoming twisted so as to lie in opposite directions to each other. He and Schaudinn are responsible for the doctrine that a copulation

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under this in their forward course. This conception of the course of the peristomal fibril is diagrammatically represented in an illustration which accompanies the article. He has evidently abandoned the view that this fibril starts from an outer pair of basal granules. In this respect the writer agrees with Rodenwaldt, but he would emphasize that he has never seen any appearances which would suggest that in adult organisms at any rate the fibril in question is continuous in its posterior half and that the axostyles and postero-lateral flagella pass beneath it.

process takes place in some of the cysts (copulation cysts). v. Prowazek thus states "Occasionally it was noted that two individuals had come into apposition and had secreted a cyst wall." The four nuclei divided once more and the resultant products emerged from the peristomal border which was visible within the cyst in the form of two strongly curved lines. Subsequent changes could not be followed with certainty. Bensen's account of cyst formation (1908) was evidently influenced by that of v. Prowazek and Schaudinn. He states the following: "Cyst formation proceeds in such manner that two individuals come into ventral apposition and then encyst. Within the cyst they loose their flagella and a union of the nuclei results. Further changes have, however, not been observed." He regarded these copulation cysts with two nuclei as characteristic of the human form. Noc (1909) states that he was unable to observe either copulation or autogamy in the cysts. He points out that nuclear division within the cyst is not necessarily limited to the binary type, but may terminate in multiple division with a corresponding division of the cytoplasm. His subsequent account of what occurs is, however, somewhat confused and his illustrations are unfortunately so crude that they do not aid in the elucidation of his meaning. The "corps énigmatique" according to Noc undergoes longitudinal cleavage within the cyst and "one asks oneself whether the resulting chromatin filaments may not serve to regenerate the posterior flagella of the daughter giardias within the cyst."

Rodenwaldt (1912), as I have stated, has given the first detailed account of the structure of the human cysts. Concerning the existence of copulation and autogamy cysts, as described by earlier writers, he thinks that such an assumption "seems somewhat audacious in view of the imperfect illustrations which have been furnished." He himself is willing to admit only "that he has evidence of division into two." "Some of the earlier stages of division," as he has seen them, "resemble the pictures of autogamy cysts of various observers and some of the more advanced stages the so-called copulation cysts." An outline of Rodenwaldt's own findings follows: Cyst formation is preceded by the appearance of a dense layer of fine granules beneath the arched dorsal surface; at the same time this latter increases in size. The dorsal portion of the animal extends ventrally over the sides, with the result that the contents of the peristome become folded in more and more. Rodenwaldt assumes that the cyst wall is formed from the granules just mentioned, because they disappear completely with the completion of the process of encystment. Inside of the denser outer covering there is in every cyst a broad, light, non-staining layer which probably represents a soft plasmatic covering of the organism within. In young cysts one can clearly see two nuclei and between them the arrangement of the basal granules with their fibrillar apparatus, with the exception of the periostomal fibril which has become separated and is curled into a dense skein whose individual loops, when viewed in a single plane, only at times appear in the form of separate arched or clasp-like bodies. This entire mass tends to sag to the posterior pole. The decussation of the anterior flagella is at this stage still distinct while the other flagella are located toward the other pole and often cross each other. The ''eigenartige Körper'' assumes a fore and aft position and seems to divide into two long compact rods. Rodenwaldt suggests that these may possibly serve to direct the two new nuclei which result from the division of the original pair, toward the posterior pole, where they are later found. After that phase has been reached the ''eigenartige Körper'' disappears and reappears only when the young animal has matured to a certain stage (see Plate XXIV, Figs. 1–4).

Immediately following encystment the division of the nuclei begins, during which process the chromatin breaks up into a larger number of fragments than were present originally. Rodenwaldt states that he was unable to count the exact number. During these stages of the process the basal granules of each side (it will be remembered that Rodenwaldt pictures and describes four) move together so closely that each pair appears like a single granule. Cysts of this type are the most common and when seen from the side have no doubt given rise to the idea of copulating organisms.

As the new set of nuclei moves to the opposite pole they take with them a set of flagella. During this process the cyst assumes a roundish form. After the migration has been completed there is a return to the oval form. At the same time the dense skein of the perinuclear fibril splits into two and the two skeins move apart (Plate XXIV, Figs. 1–4). The actual process of excystment Rodenwaldt was not able to observe. Occasionally he met with cysts which were evidently degenerating and in which larger masses of chromatin could be discerned together with remnants of nuclei. Such appearances simulate pictures which may have suggested the idea of schizogony to Noc.

ORIGINAL OBSERVATIONS.

Material and methods.

My own studies, so far as the vegetative forms are concerned, are based upon material which was freshly obtained (*a*) from three individuals who had probably acquired the infestation in Maryland, (*b*) from one South Carolina case; (*c*) from a United States soldier, who may have acquired the infestation in France (where the preparations were made). Cyst material was obtained fresh from three Maryland cases, from an individual living in Washington, D. C., and from one case who had acquired the infestation in Brazil. In addition cyst material was also available from two Chinese cases. This had been preserved when fresh in 4 per cent. formalin, and was used for comparative studies of a corroborative nature only, and not for measurements.*

Permanent preparations were made by fixing wet smears in Schaudinn's solution (at a temperature of about 50°–60° C.), and subsequently staining in Heidenhain's haematoxylin, using iron alum in 4 per cent. and 2 per cent. aqueous solution as mordant and differentiating agents respectively. For the study of the flagella eosin-methylene azure mixtures were found most useful, and occasionally Mann's eosin-methyl blue mixture was also employed.

PLAN OF STUDY.

From the survey of what has already been done on the morphological study of *Giardia enterica* it is clear that an attempt on our part to furnish a description which can be used for comparative purposes will have to be based upon a critical analysis of the findings of these earlier investigators, and a comparison of their results with my own. For convenience sake we shall study the organism, both in its vegetative and its encysted form under the following headings:

1. General morphology.
    - (*a*) Form and general structure.
    - (*b*) Supporting structures.
    - (*c*) Neuro-motor apparatus.
        - (α) Nuclei and their relation to the motor apparatus.
        - (β) Origin and course of the flagella.
    - (*d*) The so-called parabasal body.
2. Biometry.

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* The writer is indebted to Dr. W. C. Boeck for the material from the French case and the individual living in Washington, D. C.

The trophozoite.

Form and general structure.—As seen *en face* the young and fully grown trophozoites appear roughly pyriform in shape, the rounded wider portion corresponding to the anterior end and the narrow portion to the posterior end. The greater portion of the anterior end is occupied by a depressed area which represents the so-called sucking cup in which two nuclei can be clearly distinguished. These structures are convenient anatomical landmarks by which we can fix the lines of maximal width, etc., and the location of the various organelles. In most specimens the line of maximal width drawn at right angles to the longitudinal axis passes through the posterior poles of the nuclei. If we denote the entire length of the organism by the figure 100, the distance from the anterior pole of the body to the line of maximal width, as just defined, represents approximately 23.5 per cent. of the total. From the extreme outer points of this line of greatest width the lateral lines approximately maintain the same distance from the longitudinal axis for about 14.5 per cent. of the total length. Thereafter the lines gradually converge; the remainder of the length corresponding to about 26 per cent. of the total. In some specimens the posterior end becomes very much narrowed, the lateral lines running almost parallel for a distance of about 2 micra, while in others, and these constitute the majority, there is a gradual convergence toward the caudal termination (see Text figure 1).

In addition to the gracefully elongate forms which may be viewed as typical of the vegetative type one now and then encounters in nearly every slide organisms whose contours tend toward the oval and in which no marked attenuation of the tail end exists. These forms the writer is inclined to view as old forms which are preparing for encystment, without wishing to suggest, however, that only old forms can and do encyst.

When viewed from the side the organism presents a slightly upturned attenuated head tip. Immediately behind this appears a suggestion of a slight depression from which point off the dorsal surface becomes markedly arched, the highest point being reached about the middle of the posterior half of the body; there is then an abrupt drop to very near the end of the tail, and a final brief convergence with the latter.

An examination of the ventral surface* shows a cup-like depression occupying almost the entire anterior portion of the body and extending nearly to an imaginary line cutting the longitudinal axis at right angles, at a point about half way between the anterior end and the tip of the tail. The cup is roughly kidney shaped, its long axis being

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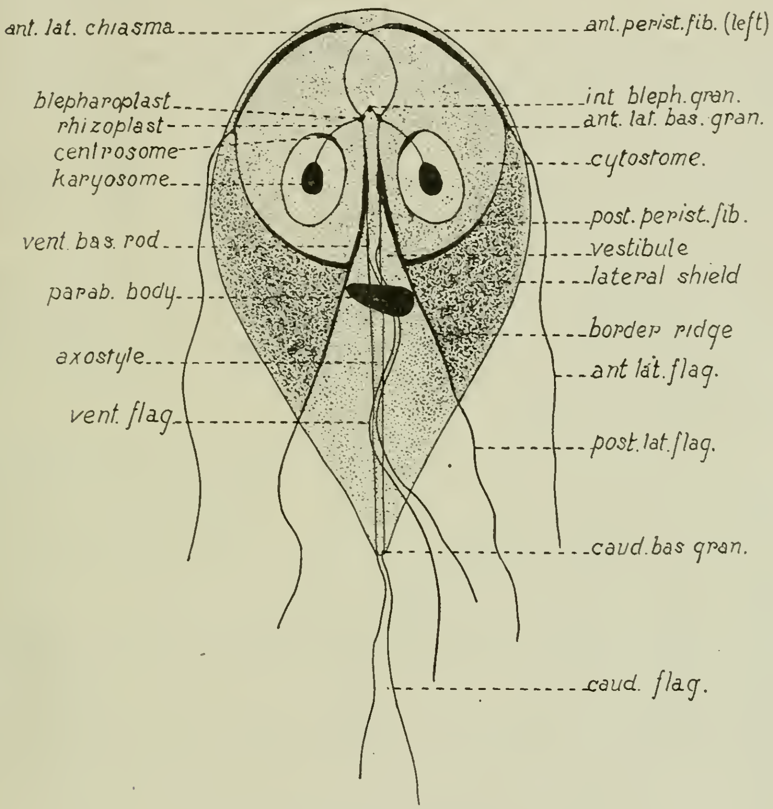
* Bearing in mind that the so-called parabasal body is located dorsally to the axostyles it is easy to determine whether we are looking at the dorsal or ventral



Text Figure 1.

at right angles to the long axis of the body. At its hilum we meet with the anterior portion of a diamond shaped depressed area which is bordered at each side of its anterior half by a triangular shield-like ventral incurvation of the dorsal surface of the animal (see Text figure 1). The diamond shaped area just mentioned evidently becomes continuous with the cytostomal depression, by way of the hilum which latter region I would propose to name the cystostomal vestibule.

surface by focussing down slowly and noting whether the plane of the parabasal body or that of the axostyles appears first.

Lying in the depth of the cytostome apparently there are the two nuclei with which are connected, in a manner presently to be described, the four pairs of flagella. The two anterior flagella—the antero-laterals—become free at a level corresponding closely to a line drawn through the anterior poles of the nuclei. The postero-laterals emerge laterally a little more than half way between these points and the tail end of the animal. The caudals emerge from the extreme posterior end. The fourth pair, the ventrals, originate apparently in the cytostomal vestibule in a manner which will be described below, and seem to become free at once. The "mysterious body," or parabasal body, as it is now called, lies a little posterior to the vestibule, within the cytoplasm between the ventral and dorsal surfaces, as may be seen in side views of the organism.

The cytoplasm in stained preparations presents an alveolar rather than a granular appearance; on the surface it has become concentrated into a fairly resistant homogeneous periplastic layer. Possibly derived from this latter through a further process of condensation, are the following structures:

Supporting structures.—It will be recalled that Rodenwaldt conceived the cytostome to be bordered by a single stout fibril which he thought originated in the cytostome from a basal granule and then ran around the entire margin to end in a corresponding granule on the other side (see Plate XXIII, Fig. 3). This conception the writer believes to be untenable. In carefully destained specimens it can be seen distinctly that two deeply staining fibrils run along the anterior margin of the cytostome from the points where the antero-lateral flagella become free to near the middle of the anterior arch of the border of the cup. At this point however, there is a definite gap over which the two fibrils do not pass. The idea that the course of the fibril is continuous at this point can only have arisen from the observation of insufficiently destained specimens, or of specimens in which the cytostomal margin had contracted at the time of fixation. This latter possibility will readily be appreciated, if it is borne in mind that during the life of the organism the cytostomal aperture is undergoing active expansile and contractile movements, while the parasite is not attached to a cell. Some specimens will naturally be fixed while the aperture is contracted. But even though specimens be present in which this is expanded it is very easy to overlook the little break in the arch and particularly so when the process of destaining has not been carried far enough.

It may be mentioned at this place that Bensen lays special stress upon the width of the space intervening between the anterior arch of the cytostome and the arch representing the head end of the body of the animal. In the human form this space is so narrow that it is often difficult to separate the lines at all. Side views of the organism will emphasize the same feature.

As I have just mentioned the two anterior peristomal fibrils pass outward to the points where the antero-lateral flagella emerge. Here their course stops and they are apparently met by two corresponding fibrils which run obliquely downward and slightly inward until they meet the inner border of the triangular lateral shields referred to above and as shown in the accompanying text figure.

It will frequently be noted that they do not reach the anterior fibrils at all, and that the gap is crossed by a much finer line. That these fibrils are not continuous with the anterior peristomal fibrils may be clearly seen in thoroughly destained specimens, providing the cytostome is well distended. It will then be noted that very often the posterior fibrils do not reach the anterior ones at all, but that there is a little gap where the cytostome is evidently only bordered by the undifferentiated periplast.

Drawings of earlier investigators picture the posterior fibrils as curving inward and forward on approaching the inner border of the lateral shields, and as actually entering the vestibule. Rodenwaldt indeed, as I have already mentioned, conceives the "single" peristomal fibril as beginning and ending in basal granules in the depth of the cytostome (see Plate XXIII, Fig. 3). As a matter of fact careful examination will show that there is nothing to support this view. The posterior fibrils meet the inner border of the lateral shields almost at a right angle, and from this point off the border of the shields does not show a *sudden* increase in the thickness, as would be expected were the course of the peristomal fibrils continued, but the increase which is discernible in the course of this border is a gradual one that has begun already before the posterior fibril has united with it. As I have pointed out elsewhere, moreover, the lateral border of the shields does not extend as far forward as the basal granules in question (see Text figure 1).

The anterior peristomal fibrils, it may be mentioned, usually appear stouter than the posterior pair, which is in accordance with their greater activity, for it is essentially the movement of the anterior arch of the peristome which brings about the expansion and contraction of the cytostome. This may be observed in animal material containing both living specimens and epithelial cells of the intestinal tract. It will then be seen that in trying to attach itself the animal first hooks the anterior arch over the top of the cell and then pulls itself into position. The rigidity of the two posterior arches is evidently enhanced by the rotundity of the two lateral shields, which in turn are stiffened along their inner margin by the so-called intracytoplasmic portion of the postero-lateral flagella. An examination of these regions will show that from the points of the lateral emergence of the exceedingly delicate flagella a line of increasing thickness, as measured by the degree of staining, on each side passes along the inner border of the triangular shields, and entering the vestibule runs forward to a level with the posterior end of the anterior third of the nuclei, the stoutest portion being the pre- and intra-vestibular portion. At the points mentioned these lines which within the vestibule run a nearly parallel course seem to stop as such, but are continued forward for a short distance by two much finer fibrils. I have gained the impression that what we see of the so-called intracytoplasmic prolongation of the postero-lateral flagella no more represents such structures than do the anterior peristomal fibrils represent the antero-lateral flagella. The diamond-shaped depressed portion which extends to the tail from the vestibule is, in my opinion, demarcated from the lateral shields by a wall which rises in height from the posterior angle of the shields and reaches its highest portion within the vestibule at a point from which other structures presently to be described evidently dip down to the bottom of the cytostome. I am not prepared to positively assert that a separate detachable fibril runs along this border, for it is quite possible that there is only a thickening of the cuticle, but certain structures that one meets in encysted forms suggest this possibility. For convenience sake I would propose to call the lines in question the border ridges of the lateral shield lines.

As regards Rodenwaldt's marginal fibril—Randfibrille—which he conceives as laterally bordering the posterior portion of the body of the animal, I have not been able to satisfy my mind that such a fibril exists, while a cuticular thickening may often be observed running along the outer margin of the lateral shields and even extending to near the end of the tail. In specimens that are apparently about to encyst this line is particularly well marked; it seems to be continuous with the head arch and in some specimens curves around to the other side, crossing over the posterior surface of the tail. Such specimens look as though the animal were covered with an oval shell, like a turtle.

Rodenwaldt, who maintains the existence of two axostyles running to the caudal end of the body from a pair of basal granules lying in the depth of the cytostome between and a little anterior to the anterior poles of the nuclei, regards these as the "Hauptstützfibrillen," the principal supporting fibrils of the animal. The writer does not share this view. The writer, like Rodenwaldt, maintains the existence of two "axostyles," but in his estimation these are too delicate to serve as supporting structures, and he looks upon them purely as true intracytoplasmic continuations of the caudal flagella. In some specimens, particularly such as have not been sufficiently destained, the caudal halves of these structures are seen in close apposition and may then suggest a single body, but from the level of the parabasal body forward into the vestibule one can *always* see two fine lines which pursue a parallel course, the interval between which is always clear and distinct. In most thoroughly destained specimens there is no difficulty in distinguishing the course of these two lines posteriorly where they end not in one, but in two basal granules. (We shall revert to this question a little later on when discussing the neuromotor apparatus in detail.)

To recapitulate, the writer would look upon the periplast of the organism as an important supporting structure in itself. This is thickened along the inner and possibly the outer border of the lateral shields, and to some extent along the lateral border of the tail. Derived from it, no doubt, are the four separate fibrils which run along the border of the cytostome, viz., the two anterior and the two posterior peristomal fibrils. These are separate and apart from the neuromotor apparatus, which we shall now consider.

The neuro-motor apparatus.—The neuro-motor apparatus of the organism is composed of two nuclei, a number of centrally located basal granules connecting with the nuclei by means of rhizoplasts, four pairs of flagella, and peripherally located basal granules (see Text figure 1).

The two nuclei are seen in the depth of the cytostome. Their anterior poles converge slightly toward the longitudinal axis of the animal. Seen *en face,* as well as from the side, they are oval in form. The chromatin in the resting nucleus is gathered into a round or oval karyosome which is united to a fairly large somewhat crescentic appearing mass of chromatin, which is apparently attached to the anterior pole of the nuclear membrane by means of a rhizoplast and which may be viewed as a centrosome. More rarely a little mass of chromatin is seen at the posterior pole or in the lateral wall of the nuclear membrane. The membrane is always distinct. A little anterior to the level of the anterior poles of the nuclei and between the two are two deeply staining granules which in some specimens can be seen to be united by rhizoplasts to the centrosome of the corresponding nuclei. They are, moreover, united with one another by a little arched commissure which carries a small granule in the center and which I would term the interblepharoplastic granule. The two outer granules are viewed as blepharoplasts. Arching out from them and running forward to cross near or in the little central gap, between the anterior peristomal fibrils, are the anterior flagella. At the point of crossing—the chiasm—a tiny granule can often be discerned. From this point on their course is obscured by the peristomal fibrils. They then emerge laterally where the latter end, and at times a little granule may be seen at the point of exit from the body of the animal. The free flagella are very delicate and can usually not be seen distinctly throughout their course in haematoxylin preparations. With Giemsa's stain, however, they can be brought out very well, and in such specimens it will be seen that they are fully as long as or longer than the entire body of the parasite. Running backward from the blepharoplasts for a distance corresponding to about the level of the posterior end of the anterior third of the nuclei are two fine parallel lines. At the level just mentioned these apparently merge with the heavily staining stout anterior endings of the border ridges of the lateral shields. From the points where these begin to diverge two fine lines run posteriorly, following a parallel course as far as the anterior level of the parabasal body, after which they gradually come closer together and finally terminate in two caudal granules, which are separated by a very small interval and from which the two caudal flagella issue. The latter are approximately as long as the entire animal. In this connection it may be mentioned that in some specimens the tail is bifid, with a granule at each end, and that in others the flagella become free a little distance before the end of the tail is reached. For the sake of convenience we may call the intracytoplasmic prolongations of the caudal flagella axostyles, but, as I have stated already, these structures seem to be too delicate to serve as supporting organs. It is possible that in the beginning of their course (reckoning from the tail end forward) they may be running along a shallow and narrow groove as far as the level of the parabasal body. This might account for the fact that in insufficiently destained specimens this portion seems to stain somewhat diffusely, and hence suggests the existence of a single axostyle. But as I have said before, the lines running forward from the level of the parabasal body are always distinctly separated with no intervening staining material of the same color whatever, and in large numbers of specimens the separate course of these fibrils can be traced to the tail without any difficulty. Kofoid and his pupils describe and picture but one axostyle as the normal in resting specimens of the animal forms which they studied in California, viz., *Giardia muris* and *Gardia microti.* According to these investigators, the axostyle starts from a single caudal basal granule and runs forward, becoming wider as it proceeds, until its termination is reached in the form of the little interblepharoplastic commissure mentioned above. When division is to take place the axostyle, according to Kofoid, splits at its broad end, with the simultaneous formation of two pairs of blepharoplasts, and gradually the split is supposed to extend to the caudal end. I have never seen any appearances suggesting such a process of division in my human material. At first I was inclined to believe that my findings were due simply to a different interpretation from that of Kofoid, but after having studied thousands of specimens I can not help but believe that my interpretation expresses the true state of affairs. Kofoid in his dividing forms pictures the commissure as broken. Whenever I have seen anything suggestive of the presence of four blepharoplasts the commissure was still intact and it appeared as though the second pair were located dorsal to the first pair. I may add that a careful study has convinced me that there is no relation between the stage of chromatinic division within the nuclei and the appearance of the two axostyles, as I have described it. As additional evidence of the normal presence of two axostyles, I may briefly record the following observation, which will be published in greater detail at another place: In the feces of one of my patients large numbers of organisms were encountered which had evidently just emerged from the cysts. In so far as it was possible to obtain a clear picture of the structures under consideration, two axostyles were seen in these forms also. Whether we have a right to call these fibrils axostyles seems rather doubtful to the writer and he would suggest that the term intracystoplasmic continuations of the caudal flagella would be more correct.

The ventral flagella apparently originate from slightly elongate basal granules or rods which seem to be attached to the axostyles about where these enter the vestibula, a little anterior to the parabasal body. They become free at once and in the living animal are engaged in a corkscrew-like motion which continues long after the lateral and caudal flagella have become quiescent. Their motion, moreover, continues while the animal is attached to a cell. They constitute its principal motor apparatus, while the other flagella execute only sluggish movements, by which the animal would be propelled only in a very indifferent manner. The ventral flagella are usually not distinct in iron-haematoxylin specimens, but may be brought prominently into view by staining with a Romanowsky mixture. Their length is somewhat difficult to measure, but apparently they extend to the end of the tail.

The postero-lateral flagella emerge at the tips of the lateral shields, which points lie a little more than half way between the caudal tip and the points of emergence of the antero-lateral flagella. v. Prowazek and Rodenwaldt both look upon the dark line which constitutes the inner border of the lateral shields as intracystoplasmic portions of these postero-lateral flagella. For reasons which I have already mentioned, I do not share this view, and it is my belief that the flagella in their course forward run along or beneath this heavy lateral line. They probably join the so-called axostyles in their intravestibular course, and conjointly with these reach the blepharoplasts. The extracytoplasmic portion of the postero-lateral flagella can be well brought out with a Romanowsky mixture and is about as long or longer than the entire body of the animal.

The "parabasal body."—This structure in the human type of Giardia usually presents a club-shaped or a heavy comma-like appearance, and in iron-haematoxylin specimens is stained black. Lateral views show that it is located dorsally to the "axostyles." Its longitudinal axis lies obliquely to the longitudinal axis of the organism, the narrow end slanting toward the posterior pole. Occasionally one gets the impression as though the structure were split longitudinally. A noteworthy fact is the frequent absence of this body. The writer has carefully studied the question whether any relation exists between the presence or absence of this body and the phase of the nuclei as regards evidence of beginning division, and also in reference to the question whether the axostyles appear single or double throughout their course. Many hundred specimens were examined from this point of view, but as the data presented in the accompanying table show, no relation between these factors could be discovered.

Nuclear phase.	Parabasal body.	Appearance of axostyles.
1. Quiescent........	Full size	Distinctly double to caudal termination.
2. “	Almost gone	“ “ “ “ “
3. “	Absent	“ “ “ “ “
4. Eight granules in parallel rows...	Full size	“ “ “ “ “
5. Eight granules in parallel rows...	Absent	“ “ “ “ “
6. Late prophase....	“	“ “ “ “ “
7. Late prophase....	Full size	“ “ “ “ “
8. Late anaphase....	Full size	“ “ “ “ “
9. “ “	Almost gone	“ “ “ “ “
10. Metaphase.......	Full size	“ “ “ “ “

Sometimes the body in question stains deeply, at other times scarcely at all. Sometimes its length equals or even exceeds that of the nucleus; at other times it is so small as to be scarcely discernible. The body is thus evidently not of a permanent nature, or perhaps it would be more appropriate to say the staining substance is not of a permanent nature. The fact, however, that when this staining substance is present it always seems to occupy the same position suggests the continued presence of a structure in which the staining substance is temporarily stored. Boeck (1919) has also come to this conclusion during his study of rodent forms. He states: “ In about half of the flagellates the parabasal bodies appeared to be situated upon or in a definite area of the cytoplasm, limited by a membrane-like structure.” This writer found that on staining with acid fuchsin and methyl green the bodies appeared red and the chromatin of the nuclei green, while in specimens stained with basic fuchsin the parabasals were not stained at all. With Wright’s stain I found that the structures took the red in the same tone as did the nuclei and the flagella. With Mann’s eosin-methyl blue mixture they were colored red, as were the karyosomes of the nuclei.

On staining Schaudinn preparations with Lugol’s solution, Boeck found that a certain number showed a mahogany colored mass “ filling all the parabasal-plasm,” and he concludes that the function of the bodies is to secrete glycogen which is retained for subsequent use in the parabasal-plasm. While the writer agrees with Boeck that the contents of the parabasals *may* be composed of glycogen, he does not believe that the iodine test alone can be viewed as a criterion of the presence or absence of glycogen. It should be borne in mind that the so-called glycogen vacuoles of certain amoebae are not stained by iron-haematoxylin.

Both Kofoid and Boeck mention that on rare occasions in some of their animal material they have seen fibrils connecting the parabasal body with the dorsal side of the axostyle. I have studied lateral views with this in mind and on one occasion thought I could discern a tiny fibril coming from a parabasal, but I was unable to trace its course.

The fact that I have been able to clearly recognize these bodies in all the very small forms which I encountered on one occasion and to which I have repeatedly alluded as probably representing freshly excysted forms, unless we assume the unlikely alternative that the enormous number had resulted from multiple fission of free forms, represents additional evidence that their presence or absence has nothing to do with nuclear division.

It will have been noted that I have avoided the term parabasal body as much as possible in referring to this structure, as its acceptance would involve the recognition of its homology with the parabasal body of Devescovina of Janicki (1911) and with the chromatic basal rod of Trichomonas, as was first tentatively suggested by Alexeieff (1914), and as apparently is held by Kofoid and Christiansen. The writer does not believe that the evidence which has thus far been adduced to support this idea is sufficient, and in a later paper he expects to discuss this question in greater detail.

The encysted form.

The majority of the Giardia cysts in the stained specimen present an oval outline. In addition apparently spherical forms will also be encountered. Whether or not these are spherical in reality may, however, be questioned. It is possible that the spherical appearance may be due to the fact that we look down upon the rounded ends of otherwise oval forms.

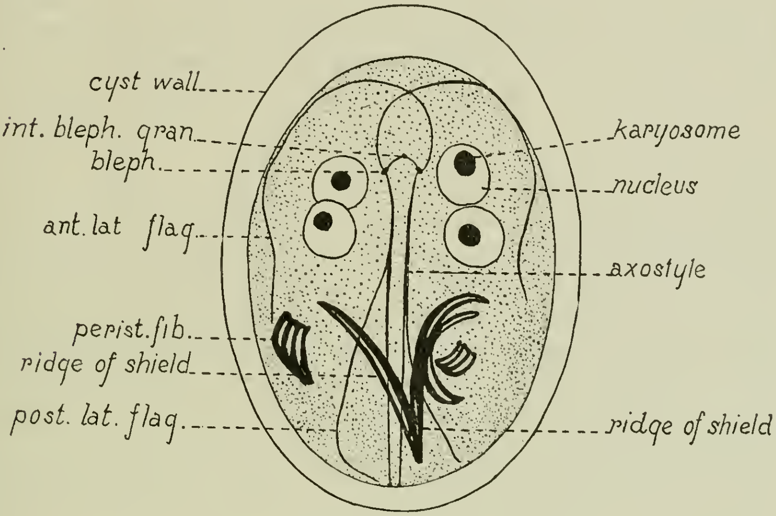
The cyst wall is apparently separated from the contents by a narrow layer of a non-staining substance. Within the cyst the organism first prepares for division and ultimately, no doubt, undergoes division, with the formation of two or more merozoites. The appearance of the contents will hence vary according to the stage of division and the number of merozoites that are being formed. An adequate mental picture of the process of division can, of course, only be formed from a study of a large number of specimens, not only from the same stool, but from stools obtained on different days, and even then it must be admitted that it is often exceedingly difficult, if not impossible, to say what this and that line in the cyst represents. It seems to be certain that at an early stage following encystment some of the structures lose their connection with the other organelles. This is true more particularly of some, if not of all, of the supporting structures. The consequence is that in most cysts one merely sees an apparently uniformly granular cytoplasm, and lying within this the nuclei and various more or less distinctly and deeply staining lines and fibrils.

In human cysts a binucleate condition is the exception. In the course of an analysis of 400 cysts from four different human hosts, 100 being studied in reference to this point in each case, the writer found that 96 per cent. were definitely qradrinucleate; the values per individual were 96, 94, 96 and 98 per cent. Binucleate cysts were rare, and a cyst containing more than 4 nuclei still rarer. I have never seen more than eight nuclei in a human cyst, and since practically all vegetative forms which one sees in the feces in the case of man are binucleate, one is forced to conclude that nuclear division must take place very quickly and very early after encystment has taken place. Preencysting forms, viz., such in which the outline of the vegetative form has become oval, are in the writer's experience all binucleate. In most specimens the four nuclei are located near one pole, usually two and two on different levels. In attempting to sketch such appearances it is hence necessary to show them as overlapping. In contradistinction to the vegetative forms the nuclei of the cysts are always spherical instead of ovoid. In their interior the chromatin will be found in various stages of division. With a sufficient amount of patience one can count eight chromosomes during one stage of the process. At one stage these arrange themselves in the form of an equatorial plate and subsequently combine in pairs, giving rise to four fairly coarse granules. The details of the process of division so far as the writer has followed them, seem to be the same as have been described for *Giardia muris* and *Giardia microti* by Kofoid and Christiansen and Boeck.

Lying between two of the nuclei one can see in most cysts two blepharoplasts still joined by their commissural fibril, at times still presenting the central granule; and running toward the posterior pole from these the two fine parallel lines that one sees in the vegetative forms. A short distance from the blepharoplasts one can then note a separation of the delicate postero-lateral flagella from the equally delicate so-called axostyles. In some specimens corresponding to this stage of development one can also see the anterior flagella, follow them after they have crossed, and observe them running down along the outer margin of the granular contents of the cyst. It is noteworthy that there are no lines in the cysts comparable to or in the position of either the anterior peristomal fibrils, or the posterior peristomal fibrils or the fibril-like structures which border the lateral shields. The ventral flagella I have at no time been able to see or identify in the cysts. At the end of each axostyle a basal granule is not infrequently discernible, and I have also seen granules which correspond in position to those from which the antero-lateral flagella emerge from the body.

In other specimens the blepharoplasts have apparently divided in a dorso-ventral direction, but even then the commissural fibril can yet be made out. A splitting of the fibrils running to the posterior end I have not been able to observe with certainty, at least not near their points of origin, though toward the far pole I have at times counted as many as eight delicate lines. More often the lines are so closely matted together that it is impossible to count them.

In addition to the structures that have been mentioned above one can see in practically every cyst certain deeply staining heavy lines. These may be divided into two groups. One group is represented by relatively short curved fibrils which on careful examination may be seen to be arranged in sets of four, each fibril terminating at one end at least, and the writer believes at both ends, in a fairly deeply staining granule. Sametimes one sees but a single set of these fibrils and at other times a double set. Rodenwaldt views these as liberated peristomal fibrils, but he evidently failed to recognize their number and separate existence and the terminal granules. The second set probably has resulted from the original set through longitudinal division. The second group of heavily staining fibrils is usually represented by two relatively long, slightly arched, fusiform, rod-like structures which are located in the posterior half of the cyst and flare out, the one to the right and the other to the left as shown in Text figure 2. Rodenwaldt suggests that these structures represent the ''mysterious body'' (parabasal) and that they may possibly serve to direct the two new nuclei, which result from the division of the two original structures, to the posterior pole. He adds that after that phase has been reached these bodies disappear, to reappear again only when the young animal has matured to a certain stage. The writer does not share this view, and prefers to leave the origin and nature of these rods undecided. His inclination is to view them as in some manner connected with or representing some of the supporting fibrils in so far as they are not represented by the smaller curved fibrils described above. Definitely opposed to the idea that they are derived from the parabasal body is the fact that with Mann's stain they do not take a red color as does the parabasal body of the trophozoite.



TEXT FIGURE 2.

Of the fate of the "mysterious body" we are in ignorance. If as Boeck suggests this structure merely represents a mass of reserve food material—glycogen—it would seem natural that this should be used up while such active changes are taking place, which involve the disorganization, division and reorganization of the organism.

In the very young forms to which I have referred above and which I am inclined to view as excysted forms the "mysterious body" could be made out, whenever the posterior end of the animal was sufficiently developed as to afford a clear view of the region which is occupied by this structure. One might accordingly question the disappearance of this body in the cyst. The writer can only say, however, that he has seen no structure which could be interpreted as such within the cyst, and it is certainly quite possible that the young organism may elaborate a reserve of food material as soon as it gets the opportunity. In some of the youngest excysted forms that I have seen there appeared to be attached to the ventral surface of the animal a delicate bud-like mass of granular cytoplasm. Whether this may represent a little store of food material with which the organism has started upon its career I do not know.

The fate of the parabasal body in the cysts I have not been able to determine. Boeck (1919) in describing his findings in the cysts of *Giardia microti* states that "In the cyst the bodies may be two or more in number and are greatly hypertrophied at the beginning of encystment. In the cyst the bodies are located in various regions, but always in the posterior part of the body. They often spread out like the tail of a comet or they may appear as conglomerations of a material, cloud-like in character, through the cytoplasm. Such was the condition of the parabasal bodies during multiple fission in *Giardia muris* (Kofoid and Christiansen, 1915)." From a study of Boeck's illustrations it is clear that he regards as parabasals those structures which the writer, as well as Rodenwaldt, looks upon as peristomal fibrils in various stages of division. Considering the normally small size of the parabasals it is difficult to conceive how the store of food material which they represent can possibly hypertrophy at a time when the animal can only break down what it has, but certainly not form more anew. Boeck pictures quite correctly the splitting up into fibrils with a granule at each end of these structures which at one phase of their development resemble the arched tail of a comet, but this splitting up would be a useless performance, if in the end they should merely disappear, and be it remembered that in his as well as in Kofoid's account of the structure of the cysts no reference is made to the fate or origin of the supporting structures.

I am quite willing to admit that I have not as yet been able to furnish the actual proof that the brush-like structures which Boeck looks upon as parabasals are in reality peristomal fibrils, but believe that the likelihood of such being the correct interpretation is greater than the probability of their representing hypertrophied parabasals. A food reserve certainly does not increase in quantity during starvation.

In conclusion I would state that I have never seen cysts derived from human beings in whom two fully developed organisms could be recognized within the cyst wall, which is so common in the case of the feces of meadow mice harboring *Giardia microti.* I have never been able to observe any further development in the cysts of *Giardia enterica* after they had been voided.

In the past it has been tacitly assumed that excystment does not occur in the digestive tract of the same individual, unless auto-infestation takes place. In the one case to which I have repeatedly referred in which what appeared to be immature forms were present in very large numbers, active movement was noted within many of the cysts in the freshly voided stools and demonstrated in the laboratory. This would suggest that the small forms which were present in the same stool were probably young excysted organisms and that excystment can actually occur within the digestive tract. Such an observation, however, is certainly exceptional, and it may be questioned whether it represents a normal process. Fully developed forms within the cyst wall were not seen in this case.

BIOMETRY.

In as much as earlier observers have given widely different measurements for the human type of Giardia, but without regard for the requirements of modern biometry, and in as much as the study of size of these low forms of life often plays an important rôle in inquiries regarding the existence of species, it seemed to the writer that a careful investigation of this phase of the problem was of paramount importance. The following factors were accordingly considered: In the study of the trophozoite: (*a*) the length of the organism; (*b*) its breadth; (*c*) the relation of length to breadth (BL/BB); (*d*) the total area of the body; (*e*) the length of the nucleus; (*f*) the breadth of the nucleus; (*g*) the ratio of the length to the breadth of the nucleus (NL/NB); (*h*) the area of the two nuclei combined; (*i*) the relation of the nuclear area to the body area (BA/NA).

In the case of the encysted organism (*a*) the length of the cyst; (*b*) its breadth; and (*c*) the ratio between length and breadth (L/B) were similarly determined.

The material for the study of the trophozoite was obtained, as in the foregoing morphological study, from five different individuals which have been designated in the tables and curves as Maryland I, Maryland II, Maryland III, South Carolina and French respectively. The cyst material was also obtained from five different individuals which have been designated as Maryland I, Maryland III, and Maryland IV, District of Columbia and Brazil. Maryland I and III of this series were the same individuals as Maryland I and III of the trophozoite series. The number of specimens measured from each individual case is given in the first column of the tables and in the legends accompanying the curves. Each set of measurements, whether recorded in tabular or curve form, is accompanied by the corresponding composite value. In the table the range between the lowest and the highest values is given, together with the mean which has been calculated not on the basis of the individual means, but upon the measurements of the entire series. Measurements of size are expressed in micra, while measurements of area have been computed in square millimeters and correspond to camera lucida drawings of a magnification of 1600 diameters. The ratios expressing the relation of length to breadth were obtained by dividing the length by the breadth.

In Table I are presented the measurements which were obtained in the study of the body of the trophozoite, in Table II the corresponding measurements of the nuclei, in Table III the area measurements of the nuclei, in Table IV the measurements of the cysts.

A study of the measurements of the body length in Table I shows an extreme composite range of 11 μ, viz., from 9.25 to 20.25 μ. It is noteworthy that the minimal ranges of the five forms are small, viz., only 2 μ, while the maximal range is much larger, viz., 5 μ. The range of the means, on the other hand, is also quite small, viz., 2.79 μ, the mean values for the five groups being nearer the minimal than the maximal figure, viz., 13.70 μ.

Earlier observers have given the following ranges for body length, but make no mention of the number of specimens that were measured; no determination of the mean has been previously recorded: Lambl 18–21 μ; Noc 16 μ; Perroncito 17 μ; Moritz and Hölzl 15.5–16.5 μ; Roos 15.5–17.5 μ; Salomon 10.0–18.0 μ; Piccardi 12 μ; Rodenwaldt 10.0–25.0 μ. It may be added that none of these observers mention the method of measuring which they employed, while our own measurements were all made from camera lucida drawings.

A survey of measurements of body breadth shows fluctuations within a narrower range than body length. This is what we would expect in as much as the length in *Giardia enterica* is so much greater than the breadth. The extreme composite range is here 5.25 μ, viz., a range between 5 and 10.25 μ. The mean values range between 6.31 and 7.87 μ and like the mean length favor the lower side, giving a composite mean of only 6.6 μ.

The figures given by earlier observers for body breadth follow: Lambl 8.6–11 μ; Noc 10.0 μ; Perroncito 10.0–12.0 μ; Moritz and Hölzl 10.0–12.5 μ; Roos 9.0–11.0 μ; Salomon 7.5–15.0 μ. These European forms, so far as length and breadth are concerned, thus seem to be a little larger than our American forms, though this conclusion is not

TABLE I.

Measurements which were obtained in the study of the body of the trophozoite from five different cases of Giardia enterica, together with the corresponding composite values.

	No. of Specimens.	Length of body.†			Breadth of body.†			Ratio of body length to breadth (B.L./B.B.).			Area of body.*		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I.	65	10.25	20.25	14.11	6.25	10.25	7.87	1.4	2.2	1.8	105	235	158
Maryland II.	82	11.25	17.25	14.34	6.25	9.25	7.83	1.5	2.3	1.8	105	245	172
Maryland III.	50	9.25	15.25	11.61	5.0	8.0	6.31	1.5	2.0	1.79	85	235	116
South Carolina.	75	10.25	15.25	12.70	5.75	8.75	6.94	1.4	2.2	1.8	95	195	146
French.	70	11.25	17.75	14.40	6.75	10.25	7.82	1.4	2.3	1.6	125	275	194
Composite.	342	9.25	20.25	13.70‡	5.0	10.25	6.6‡	1.4	2.3	1.84‡	85	275	156‡

* These figures represent the area in square millimeters of camera lucida drawings corresponding to a magnification of 1600 diameters.

† In micra.

‡ The composite means were obtained, not from the means of the five cases, but from the measurements of the total number.

TABLE II.

Measurements in micra which were obtained in the study of the nuclei of the trophozoite of Giardia enterica from five different individuals, together with the corresponding composite values.

	No. of Specimens.	Length.			Breadth.			Ratio of length to breadth (NL/NB).			Nuclear area.†		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I	50	1.6	2.6	2.14	1.0	2.0	1.41	1.0	2.0	1.51	7.5	32.5	17.0
Maryland II	50	1.6	2.6	2.04	1.0	1.8	1.44	1.0	1.8	0.91	7.5	22.5	14.4
Maryland III	50	1.5	3.0	2.10	1.0	2.0	1.58	1.0	2.0	1.46	7.5	32.5	16.0
South Carolina	50	1.5	2.6	2.16	1.0	2.0	1.47	1.0	2.1	1.49	7.5	27.5	16.0
French	50	1.5	3.0	2.18	1.0	2.0	1.50	1.0	2.1	1.45	7.5	27.5	16.8
Composite	250	1.5	3.0	2.14*	1.0	2.0	1.49*	1.0	2.1	1.36*	7.5	32.5	16.0*

† The area measurements are in terms of square millimeters and have reference to camera lucida drawings corresponding to a magnification of 1600 diameters.

* The composite means were obtained not from the means of the five cases, but from the measurements of the total number.

TABLE III.

Measurements of the area in square millimeters of camera lucida drawings of the body of Giardia enterica, of the two nuclei, and the ratio between body area and nuclear area (BA/NA). (Magnification 1600 times.)

The specimens were obtained from five different individuals. The composite values are also given.

	No. of Specimens.	Body area.			Nuclear area.			Ratio of nuclear to body area (B.A./N.A.).		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I	50	105	235	158	7.5	32.5	17.0	4.5	20.0	10.4
Maryland II	50	105	245	172	7.5	22.5	14.4	6.5	20.0	12.2
Maryland III	50	85	235	116	7.5	32.5	16.0	5.5	13.0	8.6
South Carolina	50	95	195	146	7.5	27.5	16.0	5.0	16.0	9.7
French	50	125	275	194	7.5	27.5	16.8	6.0	25.0	12.7
Composite	250	85	275	156*	7.5	32.5	16.0*	4.5	25.0	10.7*

* The composite means were obtained not from the means of the five cases, but from measurements of the total number.

TABLE IV.

*Measurements in micra of the cysts of Giardia enterica, from five different individuals, together with the corresponding composite values.**

	No. of Specimens.	Length.			Breadth.			Ratio of length to breadth (L./B.).		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I	50	8.0	13.0	10.55	6.0	8.5	7.47	1.0	1.8	1.41
Maryland III	50	9.0	12.5	11.0	6.5	10.0	8.24	1.1	1.8	1.33
Maryland IV	50	8.0	13.0	10.6	6.0	8.5	7.6	1.0	2.0	1.40
District of Columbia	50	9.0	12.5	10.8	6.0	10.0	8.28	1.0	1.6	1.33
Brazil	50	9.0	14.0	10.8	6.0	9.5	7.76	1.0	1.8	1.41
Composite	250	8.0	14.0	10.7†	6.0	10.0	7.47†	1.0	2.0	1.37†

* The measurements have reference to the cyst wall.

† The composite means were obtained not from the means of the five cases, but from measurements of the total number.

necessarily correct, in as much as the writers mentioned do not give any details regarding the number of specimens that were measured nor the method of measurement which was employed.

The values of the ratio between length and breadth, it will be noted, are remarkably constant, which would indicate that there is a definite correlation between the two. Figures for comparison do not exist.

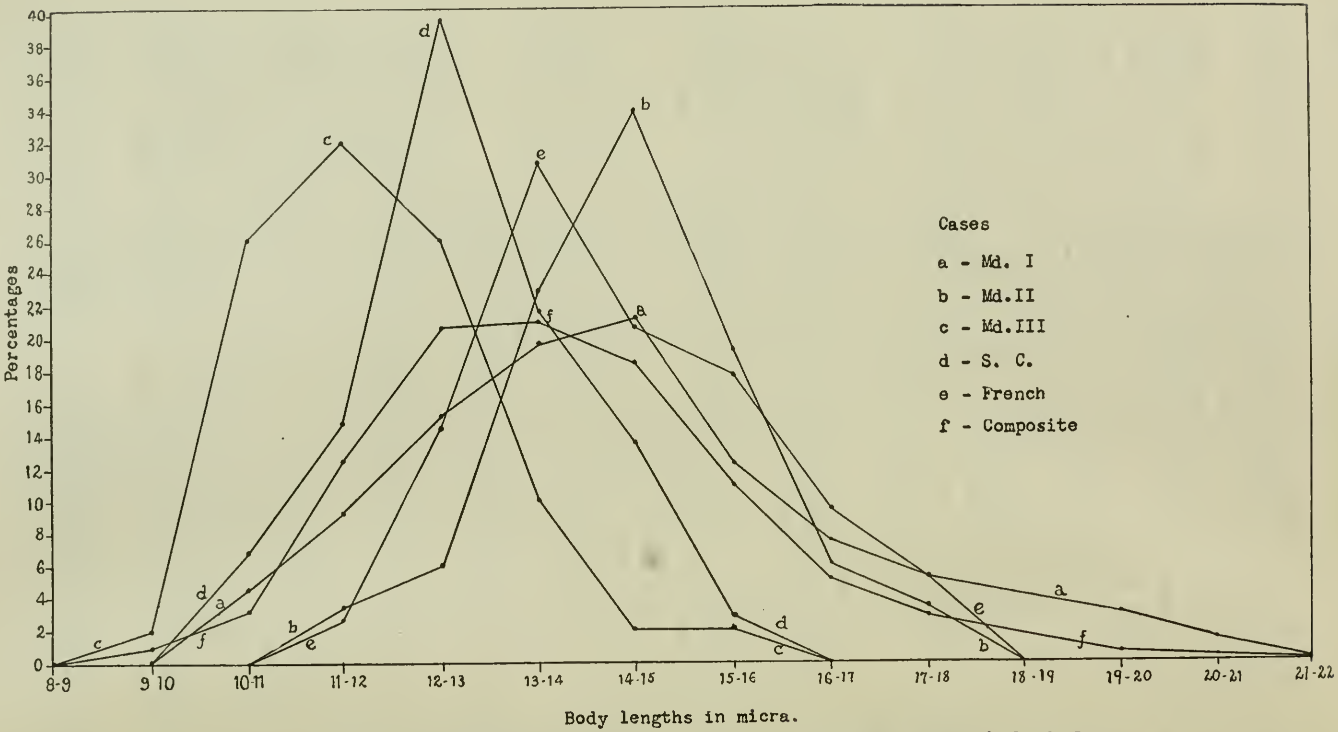
A study of the body area values and a comparison with the corresponding figures for body length and body breadth shows that the fluctuations here are no doubt largely dependent upon the variations in the former. These measurements were made more particularly for the purpose of future comparative studies and need not be further considered at this place. It may be mentioned, however, that thus far no area measurements have been published in the study of any form of Giardia.

The nuclear measurements likewise need not be considered in detail at this place; they also are here recorded only for future purposes. It will be noted that in Table II the same forms of measurements are presented as were recorded in Table I in the study of the body of the organism. The area values in conformity to the length and breadth values are very constant, which no doubt is in part due to the minute size of these structures.

In Table III the body and nuclear area values have been reassembled and their ratio to each other determined. These figures show that the area of the body as a whole is from 8.6 to 12.7 times larger than the area occupied by the two nuclei together. These values also are largely intended for future comparative studies.

In Table IV measurements of *Giardia enterica* cysts are presented. These show a greater constancy than the corresponding measurements of the trophozoite. The length range between the lowest values of the five series is thus only 1 μ, and that between the highest values only 1.5 μ, giving a mean range of only 0.5 μ, as contrasted with 2 μ, 5 μ and 2.79 μ respectively, in the case of the trophozoite. This would suggest that for comparative purposes the study of the cysts may prove of greater importance than that of the trophozoites—at least in so far as the factor of length is concerned. The composite range for length was 8.0–14.0 μ (mean 6), for breadth 6.0–10.0 μ (mean 7.47), and for the ratio between length and breadth 1.0–2.0 μ (mean 1.37).

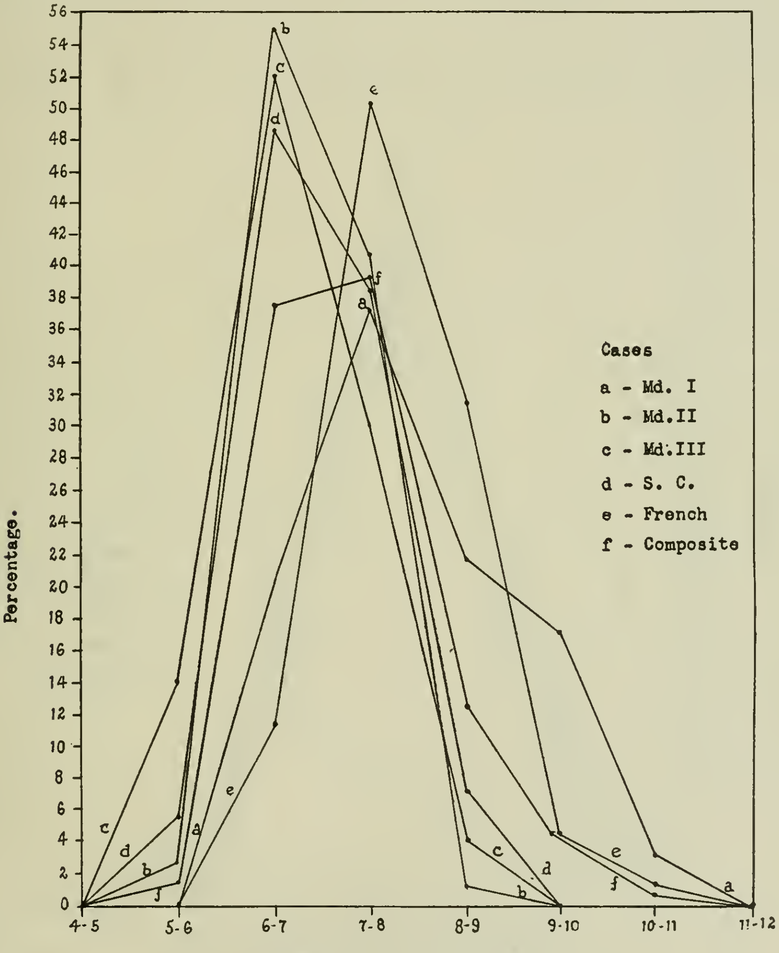
Earlier investigators have given the following values for the encysted form: Moritz and Hölzl 10.0–13.5 μ length, 10.0–10.5 μ breadth;



CURVE 1. Showing the frequency values in percentage terms, of the body length of 342 specimens of *Giardia enterica* obtained from five different individuals, together with the corresponding composite values. The individuals are designated by the letters a, b, c, d and e, respectively, and the composite curve by the letter (f). The measurements are given in micra.

Roos 9.0–12.0 μ length, 7.5–10.0 μ breadth; 5.0–7.5 μ breadth. These values agree more closely with our own, but as has been said before the earlier observers have not recorded the number of cases studied, nor the number of specimens that were measured, nor the method of measurement that was used.

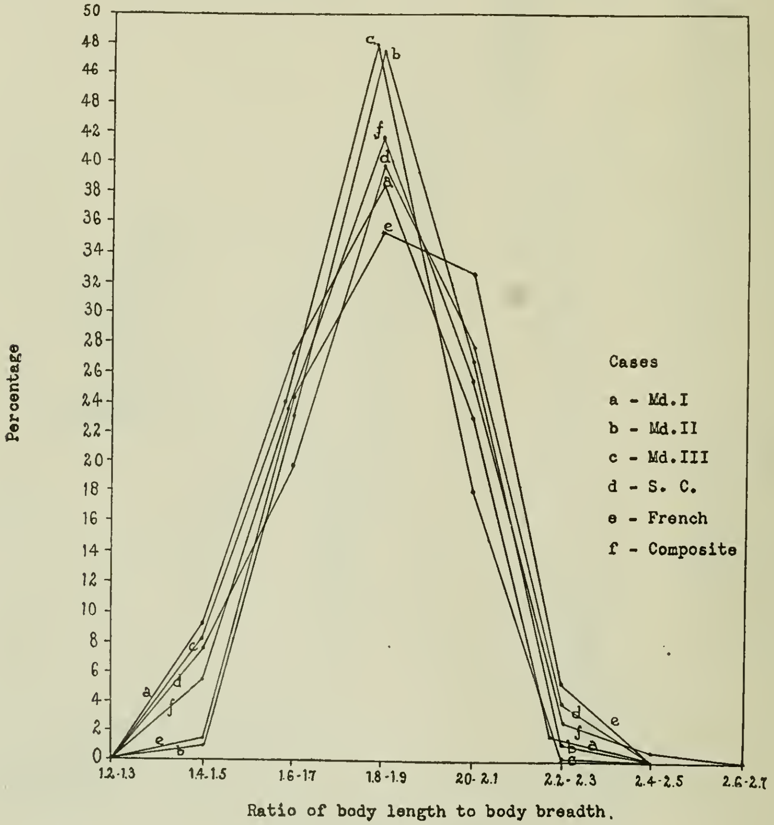
To turn now to a consideration of the various curves the writer would state that they have been introduced in this paper largely as a



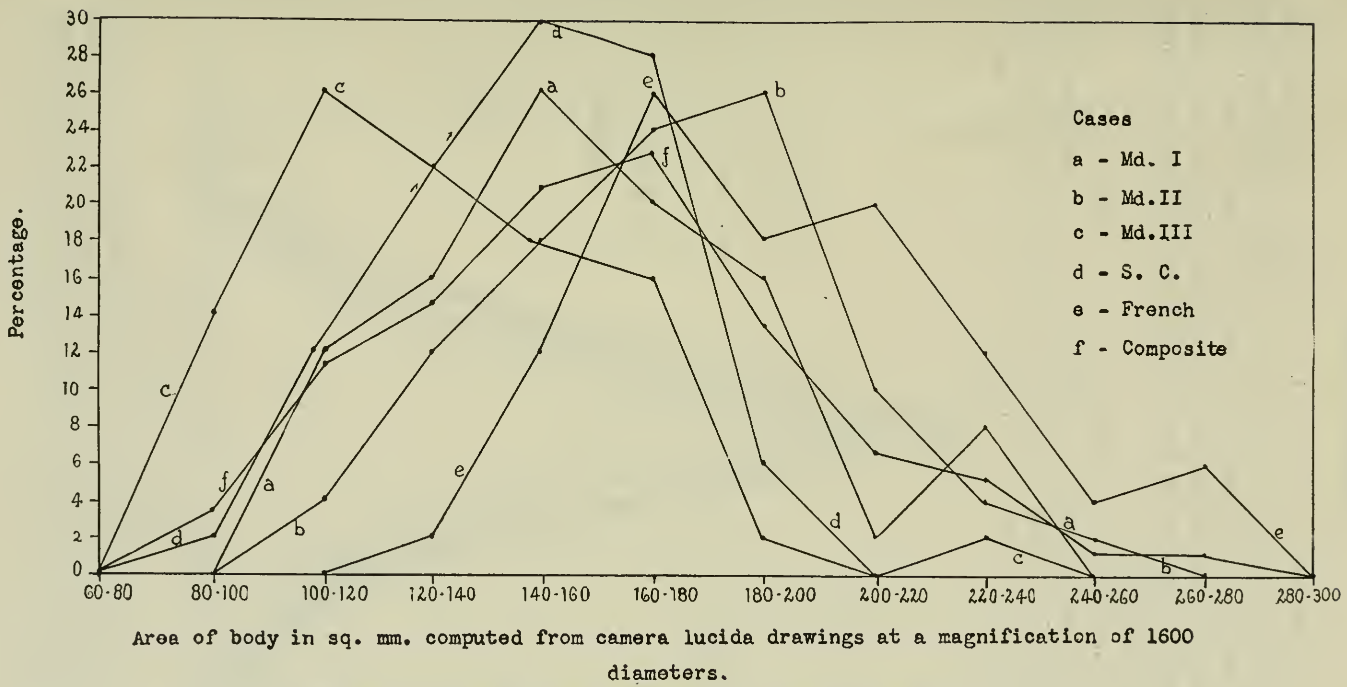
Curve 2. Showing the frequency values in percentage terms of the body breadth of the same specimens of *Giardia enterica* whose length has been recorded in Curve 1, together with the corresponding composite values (f). The measurements are given in micra.

matter of record, and to serve as basis for a subsequent comparative study of the human and the rodent types of Giardia. It will accordingly suffice to emphasize at this place only the most striking features.

A glance at Curve 1 will show that the modes expressing the length of the trophozoites from the individual cases differ but little from the composite mode, the greatest difference being noted in Maryland III where it amounts to 2 μ, while in the remaining four cases it is only about 1 μ or less. On first view of the curves one might be tempted to speak of the existence of races of *Giardia enterica,* but while such may be shown to occur, the number of specimens that have been examined for the purpose of constructing the composite curve is



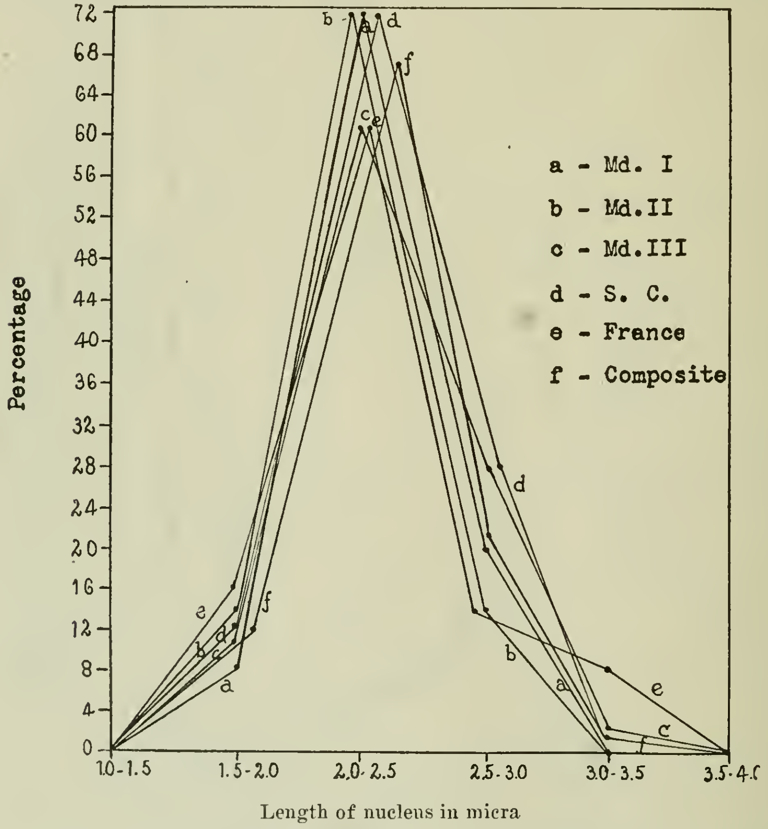
Curve 3. Showing the frequency values in percentage terms of the relation between body length and body breadth (BL/BB) of the same specimens of *Giardia enterica,* whose length has been recorded in Curve 1, together with the corresponding composite values (f).



CURVE 4. Showing the frequency values in percentage terms of the body area of 250 specimens of *Giardia enterica*, obtained from the same individuals (a, b, c, d and e), together with the corresponding composite values (f). The measurements are given in terms of square millimeters and have reference to camera lucida drawings representing a magnification of 1600 diameters.

certainly not sufficient to warrant this conclusion. As in the case of the means the modes also lie nearer the lower than the higher values. It is clear, however, that there is a considerable range in size in the length values and that this factor alone cannot play an important rôle in a study of species. A small number of measurements would have but little meaning.

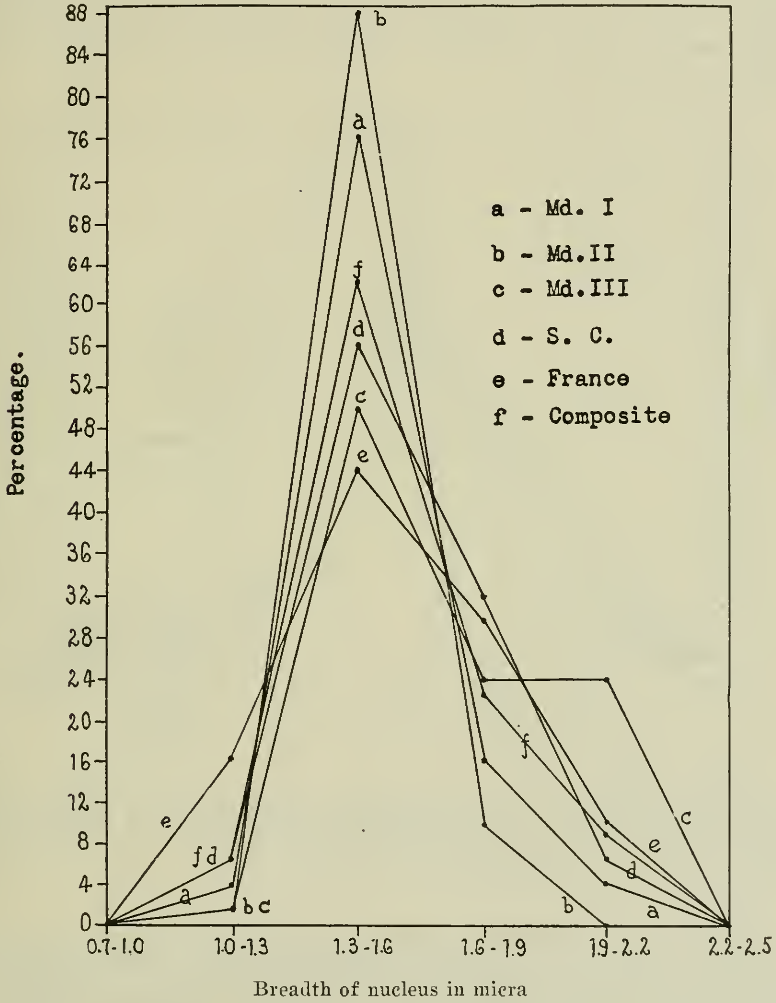
In Curve 2 are shown the frequency values in reference to breadth. It is clear that the differences occurring here are far less than those in the length of the organism, and we accordingly have much steeper and more uniform curves. The deviations of the individual modes



CURVE 5. Showing the frequency values in percentage terms of the nuclear length of 250 specimens of *Giardia enterica,* obtained from the same individuals (a, b, c, d and e), together with the composite values (f). The measurements are given in micra.

from the composite mode are here, indeed, almost negligible, amounting to only about 1 μ.

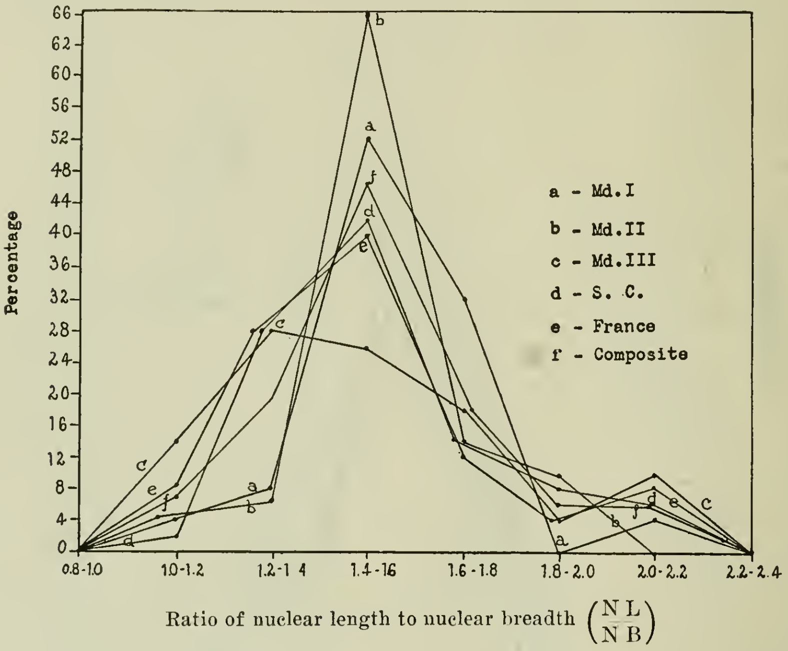
Curve 3 is particularly interesting, as it clearly shows the existence of a correlation between length and breadth, such that the one increases and decreases with the other. The modes of all five forms, as



Curve 6. Showing the frequency values in percentage terms of the nuclear breadth of the same specimens of *Giardia enterica,* whose length has been recorded in Curve 5, together with the corresponding composite values (f). The measurements are given in micra.

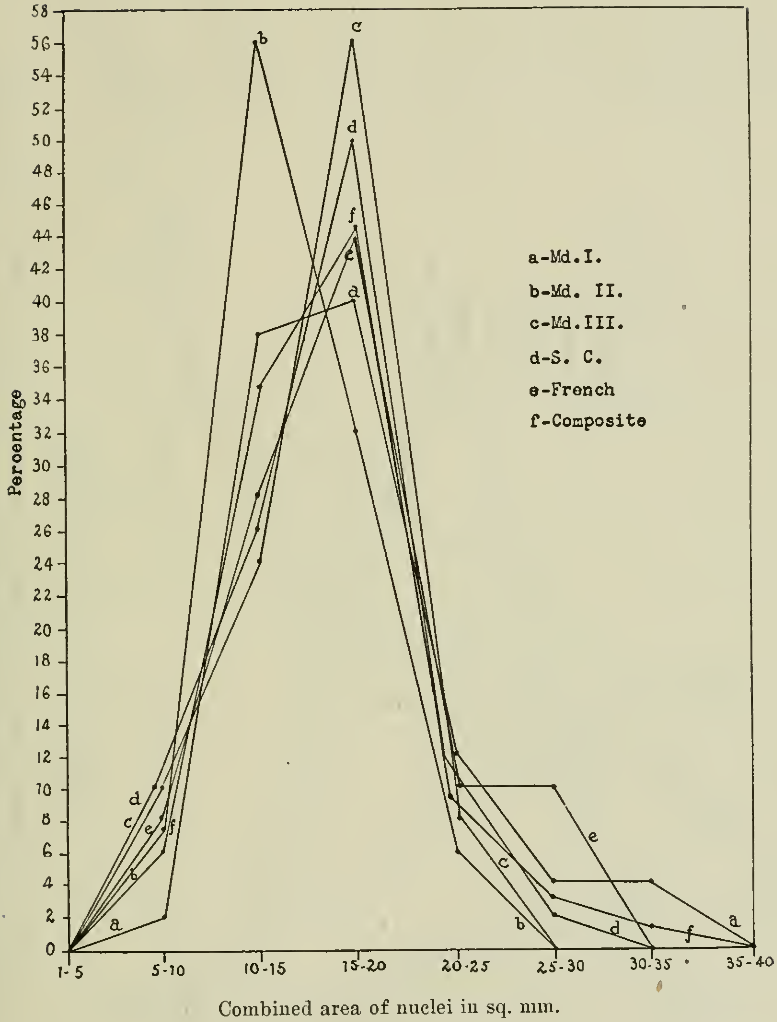
well as the composite, correspond to the same ratio between the two factors.

What has been said in reference to the variations in the length of the organism applies also to the variations in the total body area, as is clear from a study of Curve 4. The differences in the values obtained are naturally more striking than the mere differences in length. Maryland III's mode for body area, like its mode for length, is strikingly small, and one is certainly tempted to look upon this as a race differing from the rest.

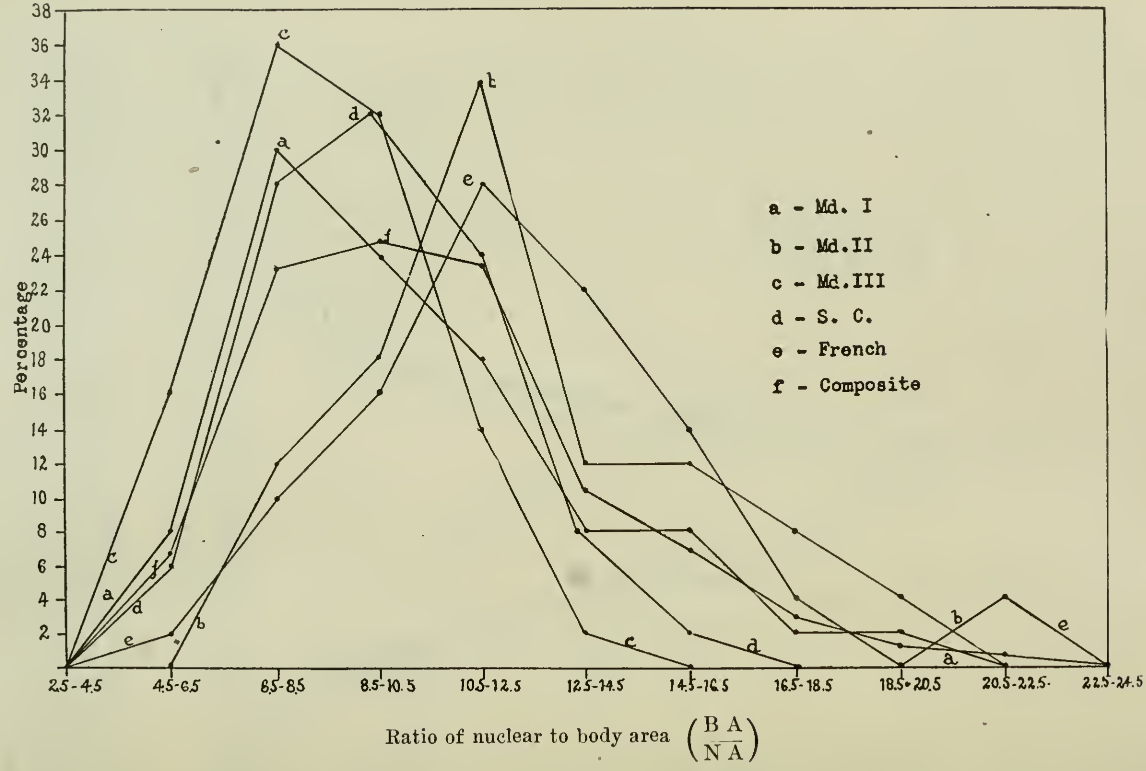


Curve 7. Showing the frequency values in percentage terms of the relation between nuclear length and nuclear breadth (NL/NB) of the same specimens of *Giardia enterica*, whose length has been recorded in Curve 7, together with the corresponding composite values (f).

Curves 5, 6 and 7 have reference to the length, breadth and the ratio between the length and the breadth of the nucleus; as would be expected in such small structures, the values show very little variation. The modes expressing length and breadth practically correspond to the same measurements, viz., to a length of 2.0–2.5 μ and a breadth of 1.3–1.6 μ. The modes of the ratios are not as high as those of the

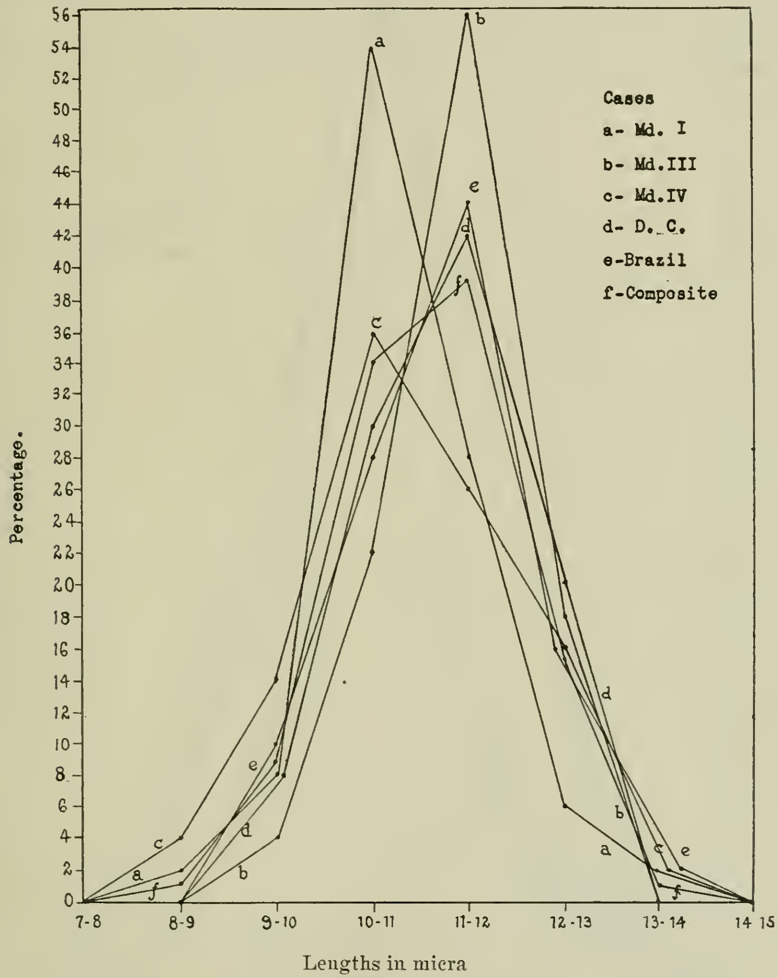


CURVE 8. Showing the frequency values in percentage terms of the area of the two nuclei of the same specimens of *Giardia enterica,* whose body area has been recorded in Curve 4, together with the corresponding composite values. The measurements are given in terms of square millimeters, and have reference to the same camera lucida drawings as in Curve 4.

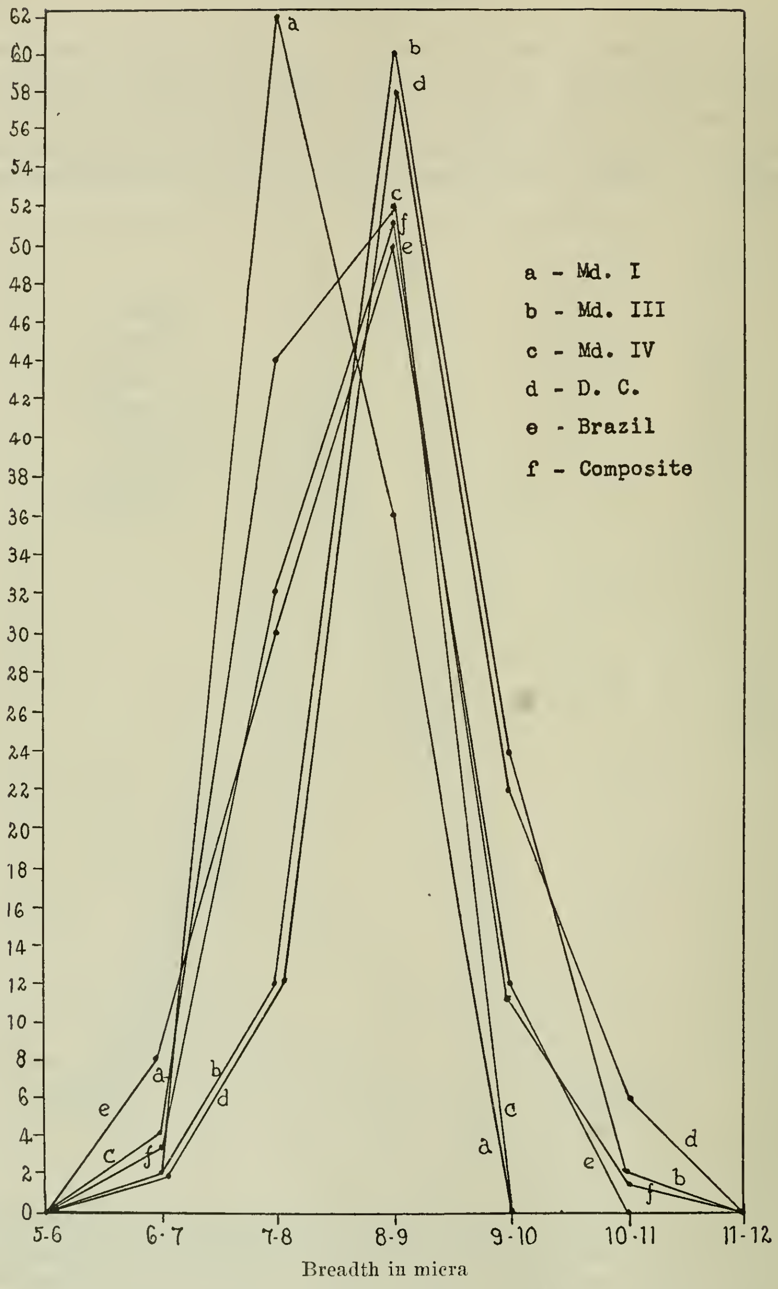


Curve 9. Showing the frequency values in percentage terms of the relation between body area and nuclear area (BA/NA) of the same specimens, whose body area and nuclear area have been represented separately in Curves 4 and 8, together with the corresponding composite values (f).

length and breadth, but they practically coincide with the composite value and correspond to 1.4–1.6. The nuclear area values—Curve 8—have reference to both nuclei combined and were determined for comparison with the total body area. The ratios between the two are shown in Curve 9. The greater deviations of the individual modes



CURVE 10. Showing the frequency values in percentage terms of the length of 250 cysts of *Giardia enterica,* obtained from five different individuals, together with the corresponding composite values. The individuals are designated by the letters a, b, c, d, and e and the composite curve by the letter f. The measurements are given in micra and have reference to the cyst wall.

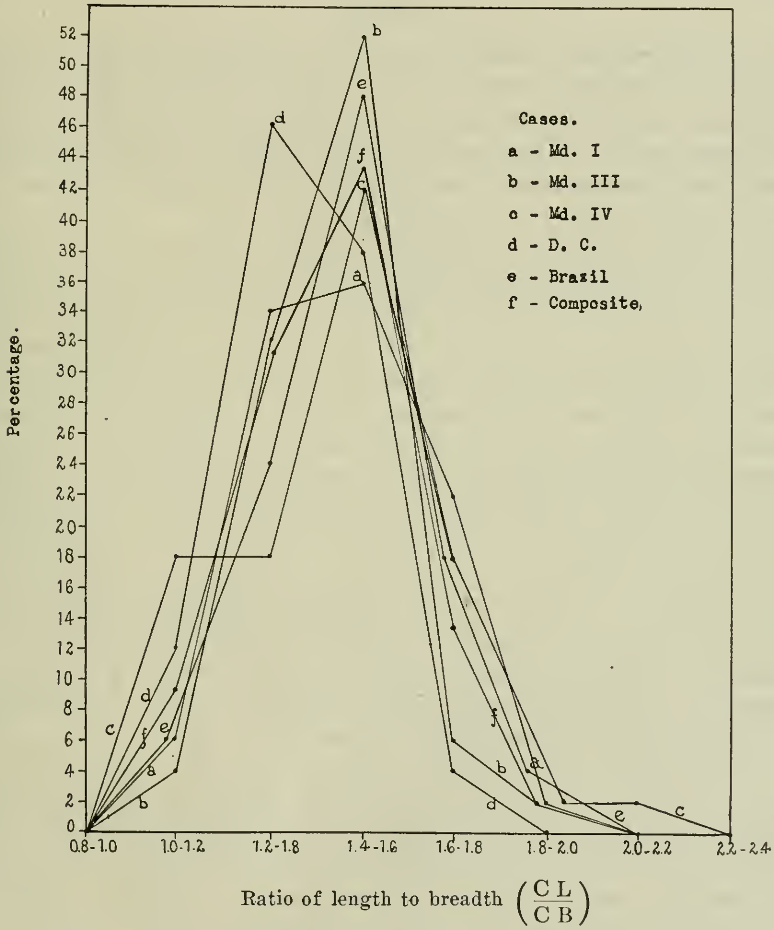


CURVE 11. Showing the frequency values in percentage terms of the breadth of the same encysted specimens of *Giardia enterica,* whose length has been recorded in Curve 10, together with the corresponding composite values (f). The measurements are given in micra and have reference to the cyst wall.

from the composite mode is evidently the expression of the greater size and variability in the length of the organism.

Curves 10, 11 and 12 have reference to the variations in the frequency values of the length, breadth and the ratio between length and breadth of the cysts.

Curve 10 shows a smaller range in the length of the cysts and a closer approach to the composite on the part of the individual modes than do the corresponding values for the length of the trophozoite.



CURVE 12. Showing the frequency values in percentage terms of the relation between cyst length and cyst breadth (CL/CB) of the same specimens of *Giardia enterica,* whose length has been recorded in Curve 10, together with the corresponding composite values (f).

The breadth values are shown in Curve 11 and are even more constant than those denoting the length of the cysts. The same is true of the ratios. The fluctuations of these latter are little more extensive than those in the case of the trophozoites. Of special interest is a comparison of the values found for Maryland I and Maryland III, which are represented in the two series. The modes for the ratios of these two forms is practically identical for the cysts and the trophozoites.

SUMMARY.

The trophozoite of Giardia enterica in ventral or dorsal view presents a pear-shaped appearance, the broad rounded portion corresponding to the anterior end and the attenuated fusiform portion to the posterior end. The ventral surface presents a cup like depression which occupies the greater portion of the anterior half of the body—the cytostome. The dorsal surface is arched.

The cytoplasm shows a delicate alveolar structure and is covered by a periplastic layer which appears denser along the dorsal surface and its triangular lateral extensions toward the ventral surface—the lateral shields. The ventral covering of the depressed diamond-shaped portion of the body between the lateral shields and extending to the tip of the tail, as well as the lining of the cytostome, appears to be more delicate. The cytostomal margin is stiffened by definite fibrils, of which there are four, viz., two anterior and two posterior peristomal fibrils. Between the anterior arched end of the organism and the anterior peristomal fibrils is a shallow groove. The anterior and posterior fibrils are not continuous with each other, leaving between them a lateral "joint." The inner margins of the lateral shields are bordered by periplastic ridges or fibrils which run forward, increasing in thickness, and reaching their greatest bulk within the vestibule, viz., the narrow passage which leads from the depressed diamond-shaped area into the cytostome.

The nuclei are two in number and located in the cytoplasm dorsal to the cytostome. They are elliptical in the resting stage and provided each with a karyosome, whence a fine linin fibril leads to a mass of chromatin which occupies a position along the anterior pole of the nucleus and possibly represents a centrosome. There is a definite nuclear membrane, but no peripheral chromatin. From each centrosome a rhizoplast curves slightly forward and inward to a granule, the blepharoplasts, of which there are two during the ordinary vegetative state of the animal. The two blepharoplasts are united with each other by a small fibrillar arch which carries a granule in the center—the interblepharoplastic granule.

The flagella are eight in number, arranged in pairs. The anterolaterals originate from the blepharoplasts. After first arching outward in their forward course they converge and cross in the "chiasm," where a granule can often be made out. They then run within the cytoplasm, apparently in the anterior groove and obscured by the anterior peristomal fibrils. They emerge from the body at the outer terminal ends of the latter, coming apparently from a basal granule. The postero-laterals likewise originate from the blepharoplasts, run along obscured by the periplastic fibrils of the "shields," and emerge where these terminate. No basal granules can be seen at these points. The caudal flagella pursue a straight though convergent course from the blepharoplasts to two basal granules at the tip of the sometimes bifurcated tail. Their intracystoplasmic portions are the "axostyles," of which there are accordingly two. The ventral flagella originate from two basal "rods" which are apparently attached to the "axostyles," one to each, at points just within the entrance to the "vestibule."

A little posterior to the point of origin of the ventral flagella and dorsal to the axostyles, within the cytoplasm, lies the "parabasal body." When fully developed this is club-shaped, and in stained preparations usually occupies an oblique position to the long axis of the body. In many specimens it is small, indistinct or apparently absent.

A biometric study of the trophozoites of *Giardia enterica* from five different individuals gave the following results:

In the *encysted state* the organism usually presents an oval, less commonly a rounded form. The cyst wall is dense, doubly contoured and highly refractive. Within the cyst wall the organism undergoes various changes associated with its division, and the appearance of the contents hence varies accordingly. In the vast majority of cysts four nuclei can be discerned; they are here round, not oval. They are usually grouped together, but in some specimens two of the nuclei may be seen "migrating" toward the posterior pole. In most specimens, even though nuclear division has taken place, only two blepharoplasts can be seen which are still united by the arched fibril carrying its central granule. The connection with the nuclei has apparently been lost. The cytoplasmic portions of the antero- and postero-lateral flagella are distinct and no longer obscured by periplastic fibrils or ridges. The "axostyles" also are seen without difficulty. Partial division of these structures is sometimes suggested in appearance, but can not be made out with certainty. The cytostome has disappeared and the periplastic fibrils (peristomal and border ridges of the shields) have lost their usual position and connection. Within the cytoplasm there appear markedly curved fibrils, each ending apparently in a granule and arranged in two sets of four each, which the writer is inclined to view as the free peristomal fibrils (evidently division of these must take place quite early following encystment). The slightly arched long and deeply staining fusiform rods which are seen in every specimen may possibly represent the freed border ridges of the shields.

The parabasal body recognizable as such is absent in cysts. Occasionally, though rarely, one meets with deeply staining coarsely granular masses scattered through the cystoplasm. Whether these are derivatives of the parabasal body is not known.

TABLE V.

Composite biometric values for the trophozoite of Giardia enterica.

Range of length.	Mean.	Mode.	Range of breadth.	Mean.	Mode.	Range of L./B.	Mean.	Mode.
A. Body:								
9.25–20.25	13.70	13.0–14.0	5.0–10.25	6.6	7.0–8.0	1.4–2.3	1.84	1.8–1.9
B. Nucleus:								
1.5–3.0	2.14	2.0–2.5	1.0–2.0	1.49	1.3–1.6	1.0–2.1	1.36	1.4–1.6

C. Area values:

Body Area.			Nuclear Area.			Body Area/Nuclear Area.		
Range.	Mean.	Mode.	Range.	Mean.	Mode.	Range.	Mean.	Mode.
85.0–275.0	156.0	160.0–180.0	7.5–32.5	16.0	15.0–20.0	4.5–25.0	10.7	8.5–10.5

A biometrical study of encysted forms of *Giardia enterica* gave the following results:

TABLE VI.

Composite values for the cysts of Giardia enterica.

Range of length.	Mean.	Mode.	Range of breadth.	Mean.	Mode.	Range of C.L./C.B.	Mean.	Mode.
8.0–14.0	10.7	11.0–12.0	6.0–10.0	7.47	8.0–9.0	1.0–2.0	1.37	1.4–1.6

In conclusion it is the writer's most agreeable duty to express his deep appreciation to Dr. Robert W. Hegner, the Director of the Department of Medical Zoology at the School of Hygiene, for his many acts of courtesy and his valuable aid and suggestions which were so freely offered in the course of the above study.

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EXPLANATION OF PLATES.

PLATE XXII.

FIG. 1. Reproduction of Lambl's first drawings, published in 1859.

FIG. 2. Lambl's more detailed illustrations of 1860. Note *g* which evidently represents a cyst.

FIGS. 3 and 4. Grassi's earliest illustrations of *Giardia enterica* (1883). Note the cyst pictured in Fig. 4*b* and the origin of the flagella in Fig. 4*a*.

FIG. 5. Bohne and v. Prowazek's illustrations of *Giardia enterica* (1908). Note their conception of the origin of the various flagella as pictured in *b*; *c* represents a cyst.

PLATE XXIII.

FIG. 1. Bensen's illustration of the type of Giardia occurring in man (1908).

FIG. 2. Two redrawn sketches from Noc; *a* represents the trophozoite, *b* a cyst (1909).

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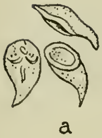

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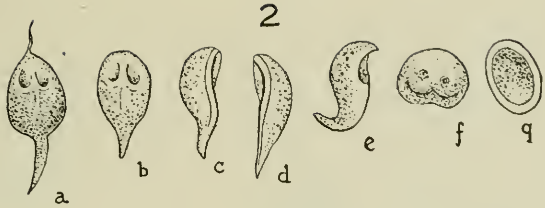

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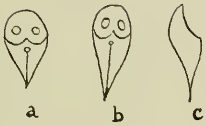

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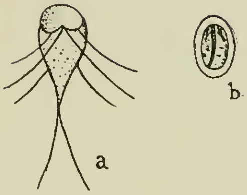

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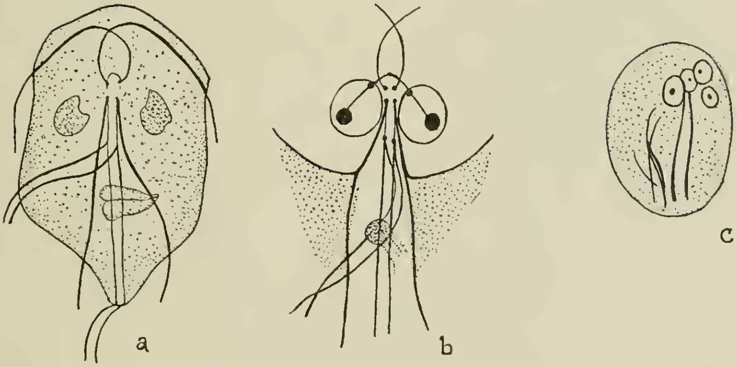

PLATE XXII.

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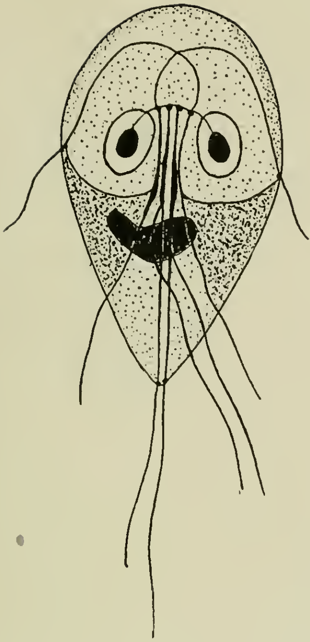

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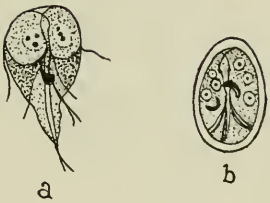

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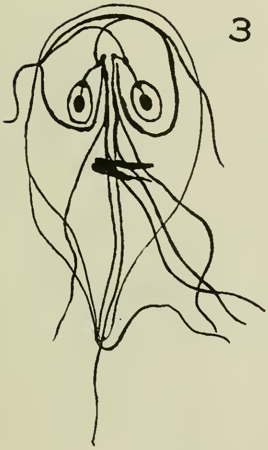

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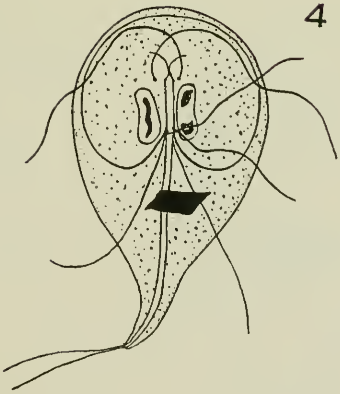

PLATE XXIII.

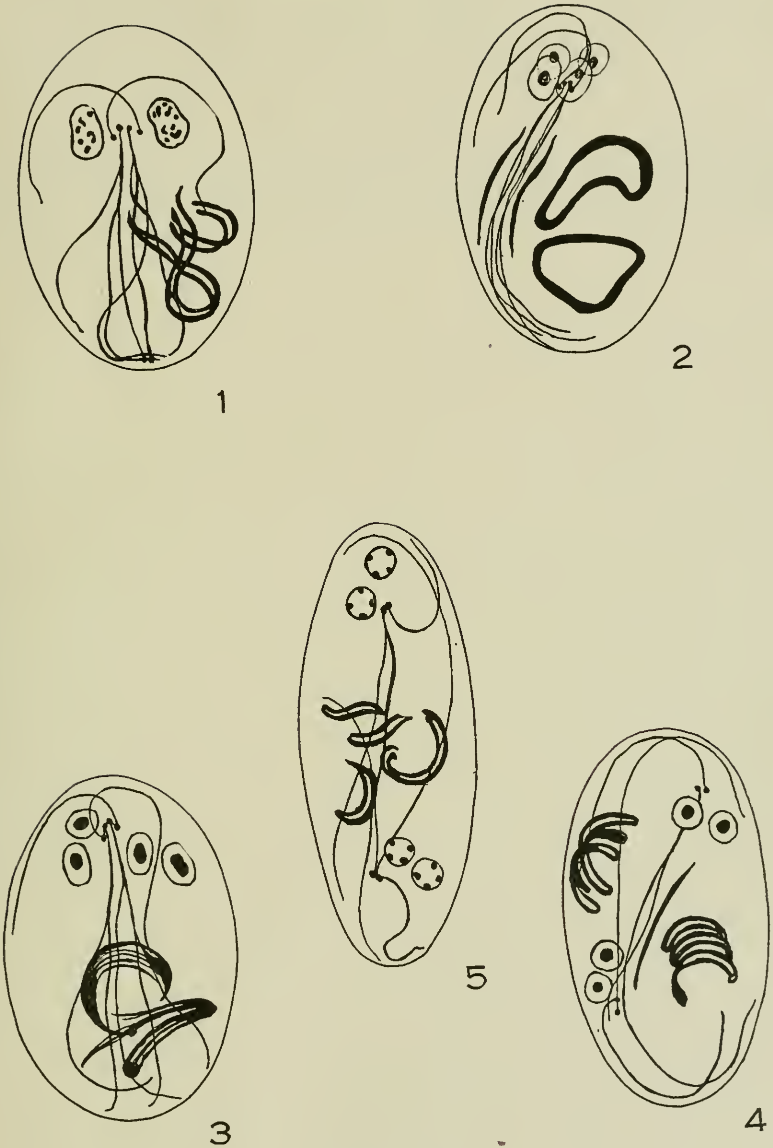

PLATE XXIV.

FIG. 3. Reproduction of one of Rodenwaldt's two indistinct drawings of the trophozoite (compare the text description) (1912).

FIG. 4. One of a pair of dividing organisms from Wenyon and O'Connor, showing their conception of the structure of the organism (1917).

PLATE XXIV.

FIGS. 1–5. Reproductions of Rodenwaldt's drawings of cysts of *Giardia enterica,* showing the division and migration of the nuclei. Note the 'skeins' representing the peristomal fibrils and the 'guiding rods' supposedly of parabasal origin (the latter pictured in 2 and 4) (1912).

STUDIES ON EXPERIMENTAL RICKETS.

VI. THE EFFECTS ON GROWING RATS OF DIETS DEFICIENT IN CALCIUM.

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(Received for publication, June 6th, 1921.)

PLATES XXV TO XXIX. (FIGURES 1–10.)

PART I.

In the first paper of this series (2) we placed on record a series of faulty diets, which, when fed to the growing rat, caused pathological conditions of different kinds to develop in the skeleton. The pathological condition induced in the bones by some of the diets bore marked resemblances to those which characterize rickets in the human being. The diets were, relatively speaking, simple in their composition; their faults were deficiencies in fat-soluble A, calcium, phosphorus, excess of calcium, etc. In an accompanying paper (3) we brought forward experimental proof of the remarkable influence exerted by cod liver oil on the deposition of lime salts in the bone of the rat. In rats which had lost the power to deposit lime salts in the epiphyseal cartilage as the result of the prolonged administration of certain faulty diets, the addition of cod liver oil to the diet without other change quickly restored the lost power to the organism. In subsequent communications we have brought forward evidence to indicate that in the presence of an insufficient amount of an organic factor in the diet—which provisionally we call fat-soluble A—a lowering of the phosphate content of the diet below a certain level—the calcium being present in optimal or above the optimal proportion, and all other constituents of the diet being in a proportion not far from the optimal—may cause changes of a pathological nature to develop in the skeleton which have all the gross and histological characteristics of rickets. When the deficiency in the phosphate content of the diet was completely compensated for, however, by the addition of the phosphate ion (the deficiency in fat-soluble A still existing) the changes which developed in the skeleton were not those of rickets, but of osteoporosis. Our experiments have shown over and over again that bone is a labile tissue, and that the character of its growth can be easily influenced through the diet, and that gross deformities of the skeleton, however much they may resemble those of rickets in the human being, can not be accepted as proof of rickets. The proof of the production of rickets must be sought in the histological changes in the bones themselves.

In papers which are forthcoming we shall attempt to describe the changes which result from restricting young rats to diets in which the fault is twofold; e.g., low calcium with low fat-soluble A, high calcium with low fat-soluble A, and low calcium with liberal amounts of cod liver oil (high fat-soluble A?). We shall also discuss the effects on the young rat of diets low in calcium, but supplemented with the vegetable oils and varying amounts of butter fat, and of high calcium with fats of various origins. In the present communication we describe the effects on growth and the changes in the skeleton which result from restricting the intake of calcium, the diet being in all other respects suitably constituted.

In interpreting the data presented in these papers it should be appreciated that modern researches in the field of nutrition have established the validity of the working hypothesis proposed by McCollum and Davis (5) in 1915. According to this hypothesis, a diet, to be satisfactory for nutrition during growth or for the maintenance of well-being in the adult over a prolonged period, must furnish the following elements or complexes. It must contain proteins in satisfactory amount. The amount of protein necessary will depend upon its quality, and its quality in turn is the result of its composition with respect to the several amino-acids which are indispensable for the construction or repair of body tissues. Proteins from different sources differ greatly in their constitution in these respects. Nine inorganic elements in appropriate compounds are indispensable. These are sodium, potassium, magnesium, calcium, iron, phosphorus, chlorine, sulphur and iodine. With the exception of sulphur, which must be supplied in the form of the organic complex, cystine, these inorganic elements are all readily assimilated and utilized in the form of mineral salts, such as the chlorides and phosphates of the alkalies and alkaline earths. The food must furnish sufficient energy-yielding substances in the form of carbohydrates or fats. The one indispensable complex in this class is glucose. Either glucose or a substance capable of yielding it must be present. The diet must likewise furnish sufficient water, and in the rat at least two still uncharacterized dietary factors now ordinarily called "vitamines." In man a third factor in this class, the antiscorbutic substance, is essential.

Much of the research in the laboratory of the Department of Chemical Hygiene during the past few years has been directed toward the study of the quality of each dietary factor in each of our more important foodstuffs of animal and vegetable origin. It has been accomplished through the application of our biological method for the analysis of a food. Following the studies of the properties of individual dietary constituents numerous experiments were carried out to determine the extent to which two or more natural foods when combined enhance each other's protein values, and mutually supplement each other's deficiencies (6).

In the course of the investigations last referred to we employed a number of diets of considerable complexity for the purpose of determining whether, when the selection was limited to such vegetable products as are functionally storage tissues of plants, viz., seeds, tubers and roots, together with the highly specialized muscle tissue of animals, a wide variety of selection could form a suitable source of nutriment. In many of the studies in this series we observed deformities suggestive of rickets. Diets composed of cereals, legume seeds and muscle meats were especially likely to induce rickets-like deformities. We came to appreciate that such food mixtures were decidedly deficient in calcium and in fat-soluble A, and to a somewhat lesser degree in phosphorus. When we undertook a systematic analysis of the nature of the factors which operated to influence the skeletal changes observed, we judged it wise first to make the analysis of those food mixtures with which we had much experience, and accordingly in the first paper of this series is described the production of rickets-like conditions by diets of a simpler character. We have continued, however, in many instances, to restrict our animals to rather complex food mixtures such as are described in this paper. The several individual factors in each of these more complex food mixtures we are now able to evaluate with considerable accuracy, and the food mixtures serve our purpose well, notwithstanding the fact that at first glance the formulas may appear unnecessarily complex and confusing. In reality they can be regarded as nearly as simple as if derived from purified foodstuffs supplemented with small additions of articles especially rich in one or another of the uncharacterized dietary essentials.

The diets employed in securing the data discussed in this paper were all fairly satisfactorily constituted with respect to all dietary factors except calcium. When casein or steak was added, the mixture was improved both with respect to protein and phosphorus. Cereal and legume seed mixtures, no matter how complex, never furnish protein of the optimal quality, and their phosphorus content is somewhat below the plane at which nutrition is best promoted. Such mixtures fall far short of meeting the need of the body for calcium during growth, and, no matter how well they may be supplemented, malnutrition will result from specific starvation for calcium unless a supplementary source of supply of it is made available. As important as phosphate additions, but far less important than additions of calcium in such diets, is an abundant supply of fat-soluble A. Diets similarly constituted, but derived in great measure from degerminated cereal products, will be much more faulty with respect to each of these factors than when entire seed kernels are used.

The animals used in these experiments were confined for the periods indicated by the accompanying charts to diets in which the main defect was deficiency in calcium. In order to avoid confusion from the possible effects of variables in the diet of a minor character, such as small differences in the quality of the protein or in the quantity of phosphorus or of fat-soluble A, we prepared the diets in such manner that these three nutrient essentials were made more abundant than in other experiments. If in such diets as we are discussing sufficient calcium is added, this will improve their biological value to an extent which can be detected in increased vitality as shown by span of life, fertility and diminished mortality among the young. Since the acidity of the seed or seed and steak mixture might affect in some way the growth of the osseous tissue, we neutralized this in some diets by the inclusion of an appropriate amount of sodium bicarbonate.

The following diets were employed:

No. 2684.

	Per cent.
Wheat	30.0
Maize	20.0
Polished Rice	12.0
Rolled oats	11.5

Peas	11.0
Navy beans	11.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	2.0

Ration 2684 is characterized by a marked deficiency in calcium. Since the ash of seeds is distinctly acid, ranging from the equivalent of 2.63 c.c. to 68.34 c.c. N/1 solution per 100 grams, we added in this case 1.5 gms. of sodium bicarbonate per 100 gms. of ration. The diet was thus made to contain a slight excess of bases over acids. For lack of calcium very little growth took place. This same food mixture with the sodium bicarbonate replaced by calcium carbonate induced optimal growth in the first generation, but, apparently owing to a deficiency of the food in phosphorus the later generations became undersized and the mortality among the young was high. On the diet of Lot 2684 the animals aged very early.

No. 2810.

	Per cent.
Whole wheat kernel	30.0
Whole maize kernel	14.5
Polished rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Round steak (beef)	10.0
NaCl	1.0
Dextrin	1.5
Butter fat	5.0

Ration 2810 was closely similar to ration 2684, except that it contained 10 per cent. of round steak. This addition improved the quality of the seed proteins in a degree which can easily be demonstrated experimentally. It also raised the phosphorus content from .3047 to .3726 gm. per 100 gms. of mixture, at the same time increasing the acidity of the diet, because of the mineral content of the cereal and legume mixture, from the equivalent of 16.61 c.c. N/1 base solution to 5.75 c.c. N/1 acid solution per 100 gms. of the ration as fed. These rats were, therefore, on the same deficient intake of calcium as were lot 2684, but had much more phosphorus and a more acid food supply. The content of the fat-soluble A in their diet was also higher than that of Lot 2684 (Chart 1), but the latter were supplied with enough of this factor to fully meet the needs of the growing rat when all other dietary factors are satisfactory.

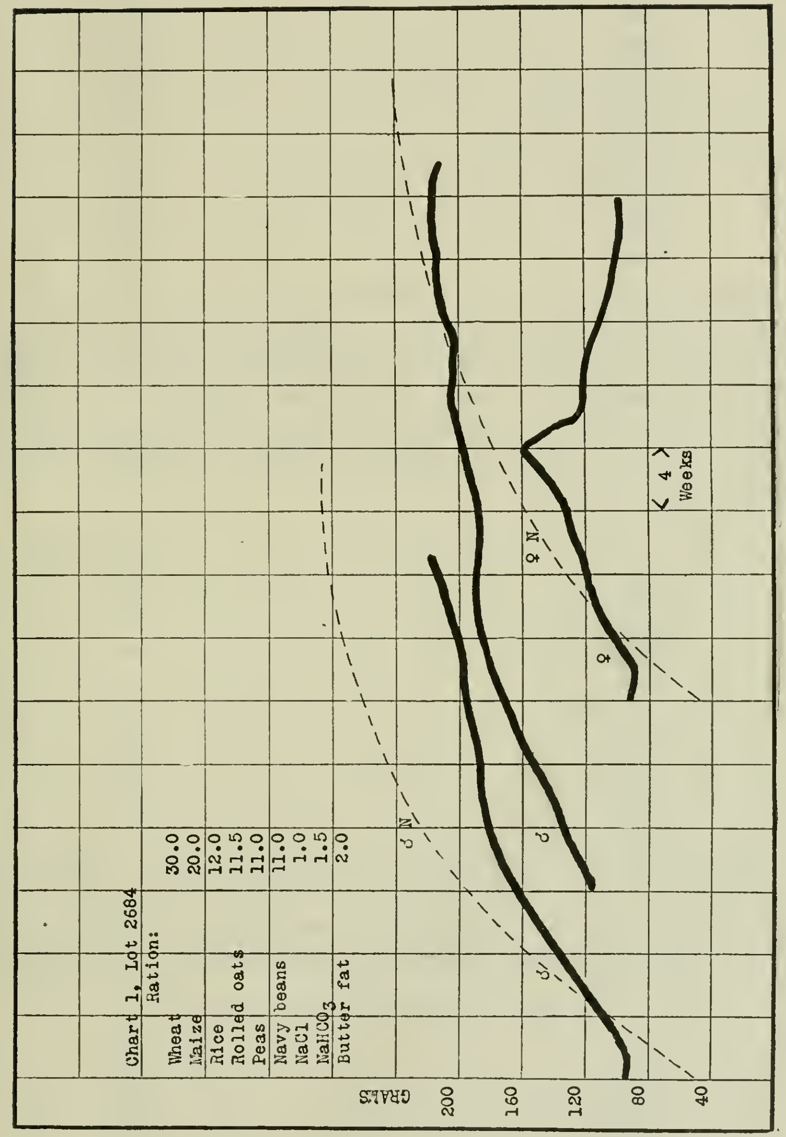
Chart 1, Lot 2684
Ration:
Wheat 30.0
Maize 20.0
Rice 12.0
Rolled oats 11.5
Peas 11.0
Navy beans 11.0
NaCl 1.0
NaHCO3 1.5
Butter fat 2.0
GRAMS
200
160
120
80
40
♂ N
♂
♂
♀ N
♀
< 4 >
Weeks

No. 2436. (Control Experimental Ration.)

	Per cent.
Wheat	30.0
Maize	19.5
Polished Rice	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Beefsteak	10.0
NaCl	1.0
$CaCO_3$	1.5
Butter fat	3.0

Ration 2436 was like ration 2810, except that the dextrin of the latter was replaced by 1.5 per cent. of calcium carbonate. This addition had a most remarkable effect in improving the quality of the diet. The animal appeared to be in the optimal condition and a family restricted to this diet appeared in the fourth generation to be entirely normal. One female of the fourth generation successfully weaned three litters (17 young).

No. 2731.

	Per cent.
Wheat	30.0
Maize	19.5
Polished Rice	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Steak	10.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	3.0

Ration 2731 was almost identical with ration 2810. It differed in containing 1.5 per cent. of sodium bicarbonate, which changed it from a distinctly acid to a slightly alkaline reaction. The animals placed upon it became short and stocky, were rough looking and died early.

No. 2435. (Control Experimental Ration.)

	Per cent.
Wheat	30.0
Maize	20.0
Polished Rice	11.0
Rolled oats	11.5
Peas	11.0
Navy beans	11.0
NaCl	1.0
$CaCO_3$	1.5
Butter fat	3.0

Ration 2435 differed in its properties from ration 2731 in three respects. The former had a lower protein content and was poorer in phosphorus, because it lacked the beefsteak which the latter contained. Furthermore, it contained 1.5 per cent. of calcium carbonate instead of sodium bicarbonate. These modifications made a remarkable difference in the growth and vitality of the animals. Lot 2435 grew normally and showed high fertility, but the infant mortality was high. They deteriorated from generation to generation, because of the shortage of phosphorus, and the relatively poor quality of proteins. This group should be compared with Lot 2436 (Chart 2).

No. 2581.

	Per cent.
Beef liver (steamed and dried)	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
Dextrin	65.0
Butter fat	3.0

Ration 2581 was of good quality with respect to all constituents other than calcium, in which it was very deficient. The acidity of this food, owing to the high content of phosphorus and sulphur derived from liver and casein, was very great, and aside from the lack of calcium was the only known fault. It is of great interest from the standpoint of the establishment of the validity of our method of interpretation of the causes of the bone lesions, discussed in these experiments, to compare the histological picture seen in animals restricted to diets having the same faults, but derived chiefly from such widely different sources as cereals and legume seeds in one instance, and liver, casein, dextrin, butter fat and salts in the other. The effects on the bones were very similar.

On the diet of Lot 2581 all rats died very early. They were short, stunted, thin animals, with serious deformities of the thoraces.

This diet modified by the addition of calcium carbonate to the extent of 1.5 per cent., and without the addition of butter fat, induced approximately normal growth and fertility. There was no evidence of rachitic deformities. All the fat-soluble A and a specific antirachitic substance, if there is such a substance, were derived entirely from 25 per cent. of liver in the diet.

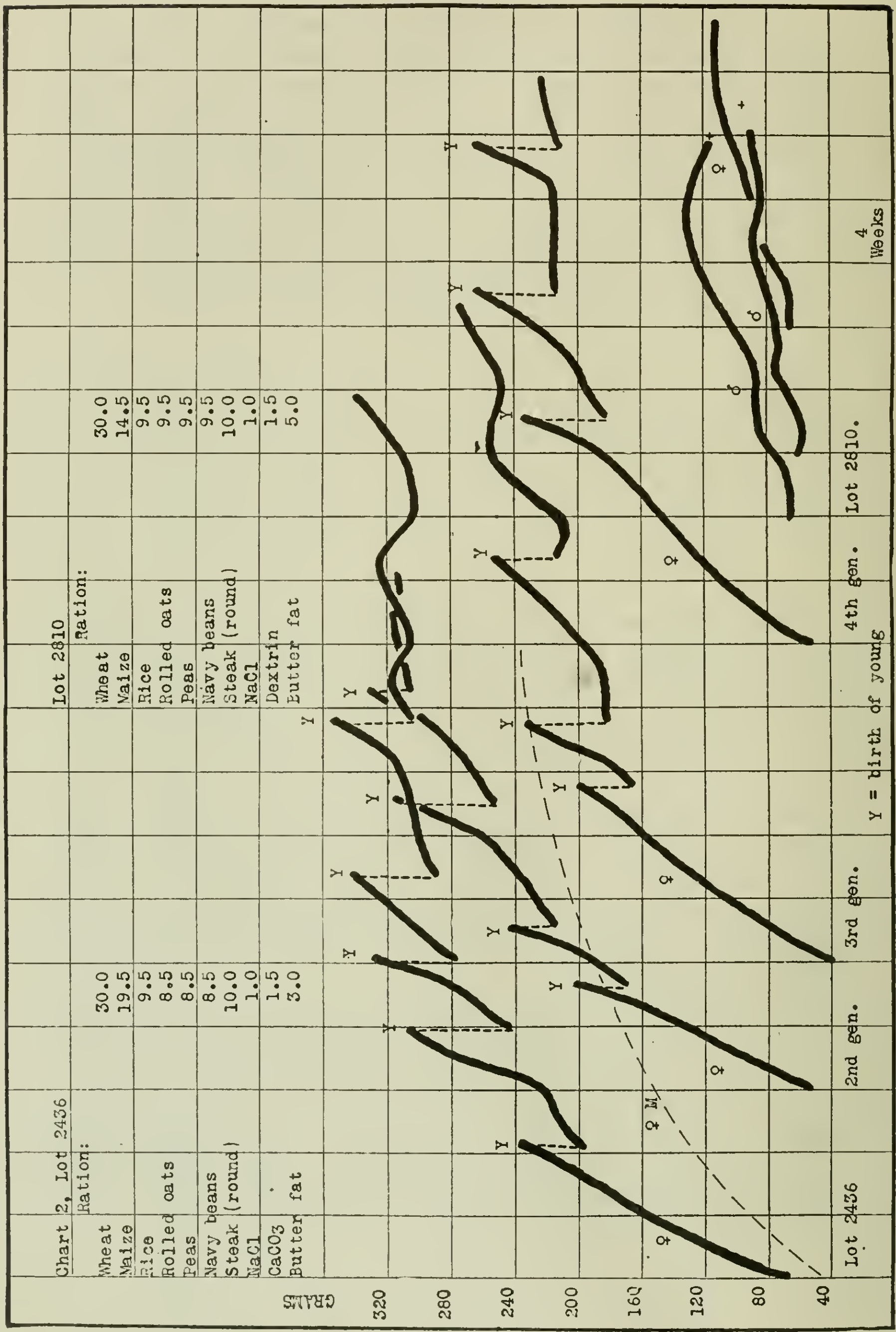
Chart 2, Lot 2436
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 8.5
Peas 8.5
Navy beans 8.5
Steak (round) 10.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
Lot 2810
Ration:
Wheat 30.0
Maize 14.5
Rice 9.5
Rolled oats 9.5
Peas 9.5
Navy beans 9.5
Steak (round) 10.0
NaCl 1.0
Dextrin 1.5
Butter fat 5.0
GRAMS
320
280
240
200
160
120
80
40
Y
♀
♀ M
♂
Lot 2436
2nd gen.
3rd gen.
4th gen.
Lot 2810.
4 Weeks
Y = birth of young

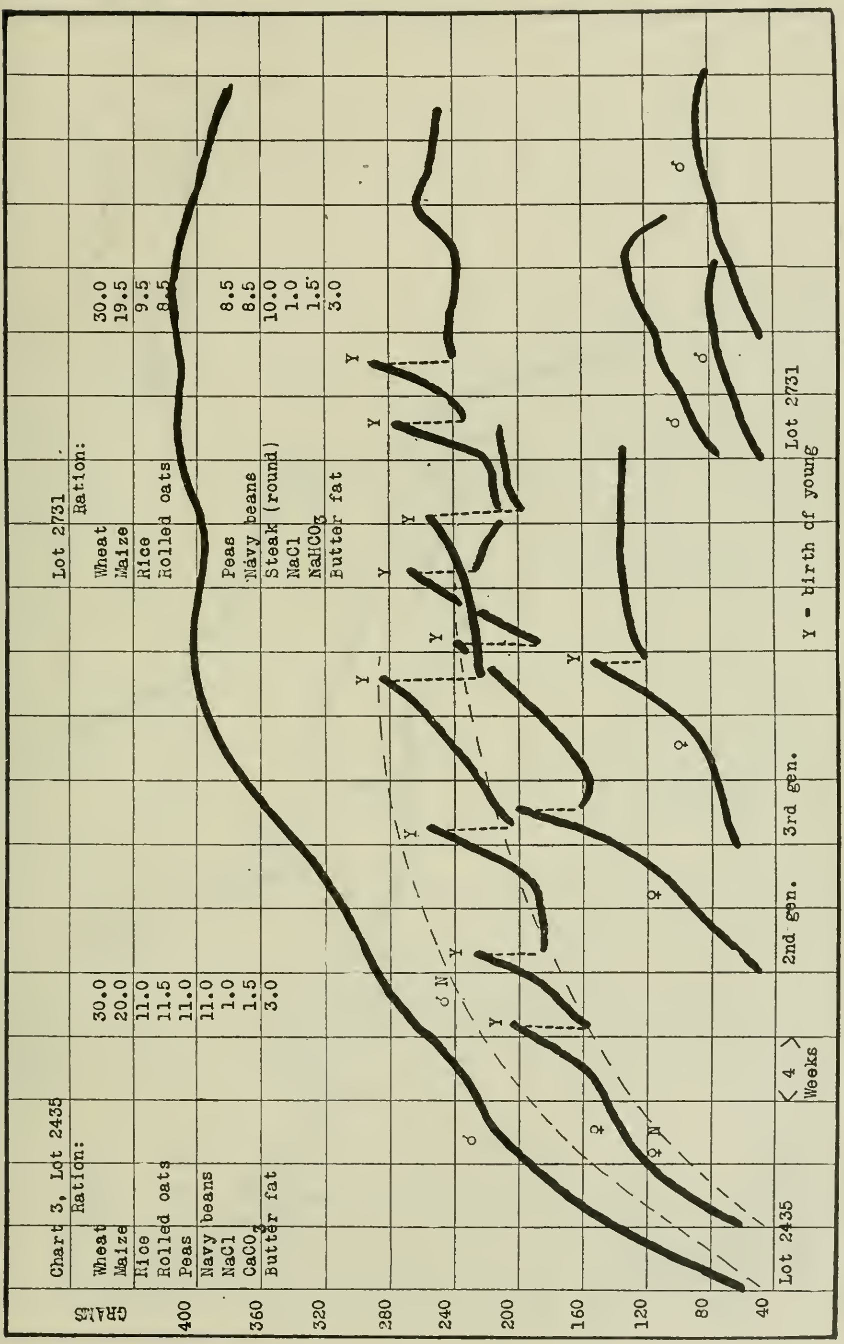
Chart 3, Lot 2435
Ration:
Wheat 30.0
Maize 20.0
Rice 11.0
Rolled oats 11.5
Peas 11.0
Navy beans 11.0
NaCl 1.0
$CaCO_3$ 1.5
Butter fat 3.0
Lot 2731
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 8.5
Peas 8.5
Navy beans 8.5
Steak (round) 10.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 3.0
GRAMS
400
360
320
280
240
200
160
120
80
40
Lot 2435
< 4 > Weeks
2nd gen.
3rd gen.
Y - birth of young
Lot 2731
♂
♀
♂ N
♀ N
Y

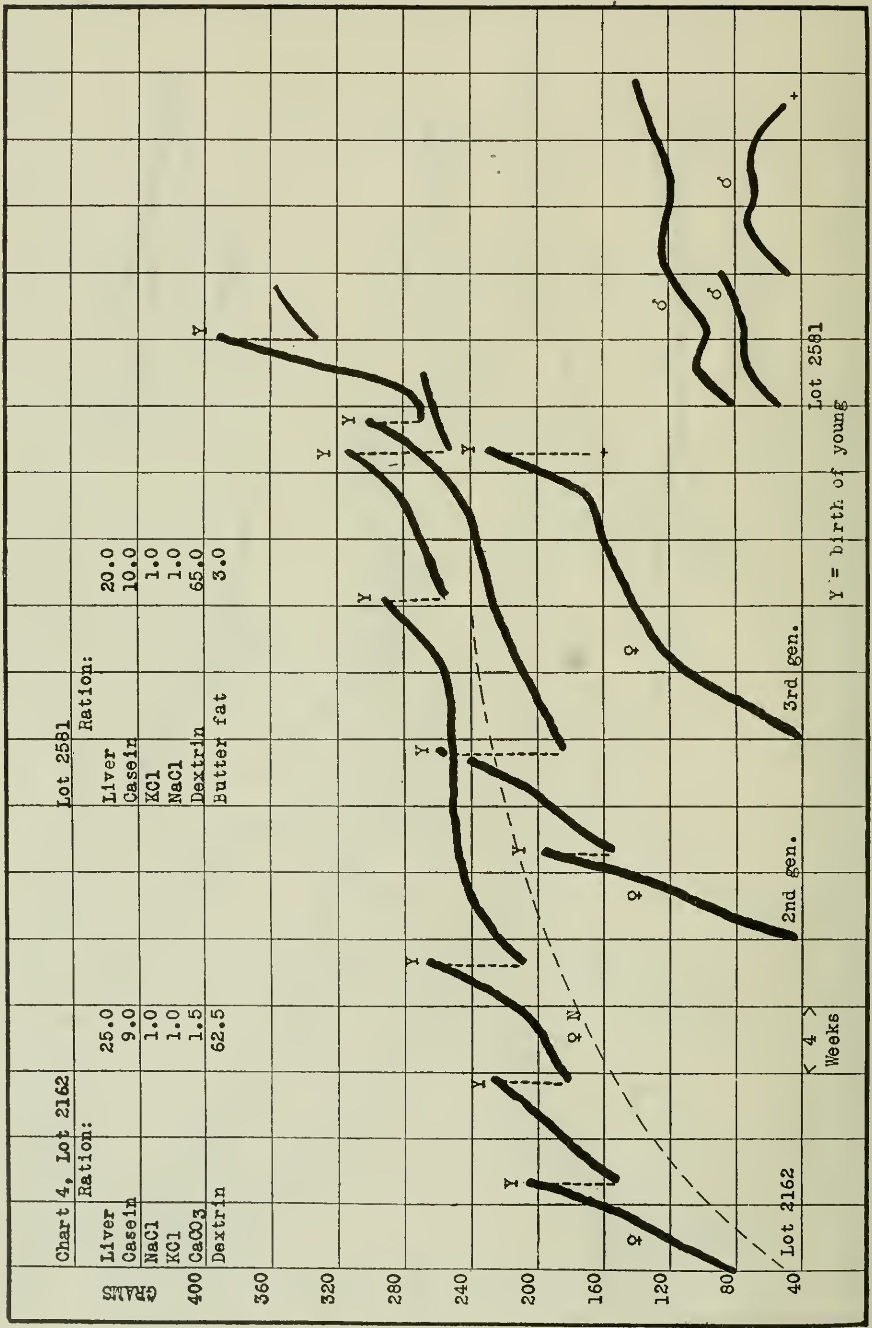
Chart 4, Lot 2162
Ration:
Liver 25.0
Casein 9.0
NaCl 1.0
KCl 1.0
CaCO$_3$ 1.5
Dextrin 62.5
Lot 2581
Ration:
Liver 20.0
Casein 10.0
KCl 1.0
NaCl 1.0
Dextrin 65.0
Butter fat 3.0
GRAMS
400
360
320
280
240
200
160
120
80
40
Lot 2162
2nd gen.
3rd gen.
Lot 2581
Y = birth of young
4 Weeks
Y
♀
♂
N

No. 2809.

	Per cent.
Liver	20.0
Casein	10.0
$NaHCO_3$	1.5
KCl	1.0
Dextrin	64.5
Butter fat	3.0

This ration was exactly similar to ration 2581, except that the sodium chloride was replaced by sodium bicarbonate. This neutralized the acidity of the former diet. That the reaction of the food within the limits of variation seen in this series of diets is not a factor of importance, the similarity in the character of the bone changes clearly show. There is no evidence that the neutralization of the acidity of the ration 2809 by the inclusion of sodium bicarbonate had any beneficial effect upon the animals. The gravity of the calcium deficiency was so great that the rats restricted to it were ruined by this deficiency alone. They developed deformities similar to those seen in the rats on ration 2581 (Chart 4).

No. 2143. (Control Experimental Ration.)

	Per cent.
Liver	20.0
NaCl	1.0
$CaCO_3$	1.5
KCl	1.0
Dextrin	73.5
Butter fat	3.0

Ration 2143 differed from ration 2809 in that it contained no casein, and all of its protein was derived from 20 per cent. of liver; and also in that it contained 1.5 per cent. of calcium carbonate. Even with the lower protein supply the animals were able, when a supply of calcium was available, to develop in a fairly satisfactory manner and to produce and rear some young. They showed no evidences of the deformities seen in Lot 2809.

PART II.

The earliest attempts to produce rickets in animals were made by feeding diets which were low in calcium. After the anatomical studies of Pommer had made clear that the fundamental lesions of the disease, in so far as the skeleton was concerned, were defects in the

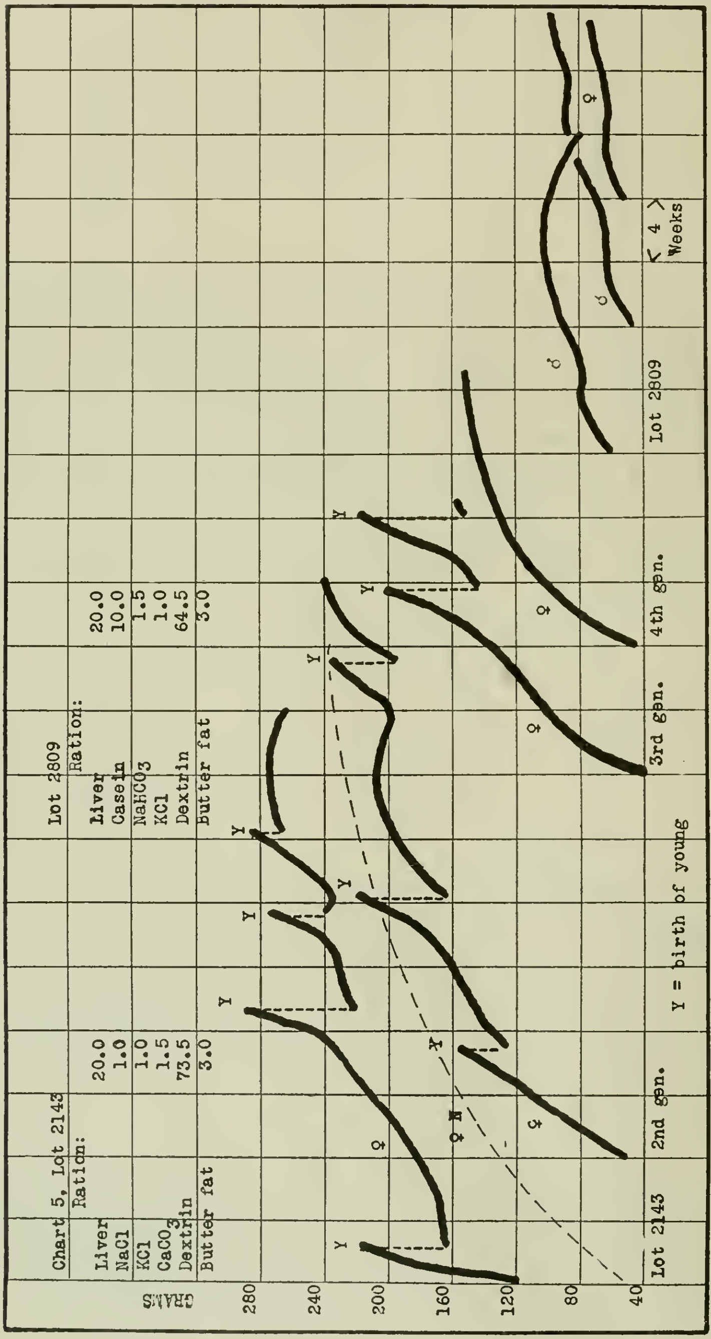
Chart 5, Lot 2143
Ration:
Liver 20.0
NaCl 1.0
KCl 1.0
$CaCO_3$ 1.5
Dextrin 73.5
Butter fat 3.0
Lot 2809
Ration:
Liver 20.0
Casein 10.0
$NaHCO_3$ 1.5
KCl 1.0
Dextrin 64.5
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
Y
Y
Y
Y
Y
Y
Y
Y
Y
♀
♀
♀
♀
♀
♀
♂
♂
Lot 2143
2nd gen.
Y = birth of young
3rd gen.
4th gen.
Lot 2809
4
Weeks

calcification of cartilage and bone, it seemed logical to conclude that a deficiency in the intake of calcium might be the cause of the disease. Numbers of investigators have made attempts to produce rickets by feeding diets insufficient in calcium salts. At the present time, however, it is difficult to know what value to place upon their work. In the first place, it is almost impossible to decide whether the changes described in the bones of their animals were or were not equivalent to human rickets. In the second place, it is by no means easy to make certain that the deficiency in calcium in their diets was the sole fault. Almost all of the work of the investigators of this particular problem was performed prior to 1910. Before that time a diet which contained protein, carbohydrate and fat in sufficient amounts to furnish the energy requirements of the body, together with a supply of certain inorganic salts, was considered adequate for the maintenance of life and normal development over any number of generations. It stands to reason, therefore, that with the increased knowledge of today the recognition of dietary defects is easy in formulas which 15 years ago passed as deficient only as regards their content of calcium. A diet used by one of the investigators in question will suffice as an example. Dibbelt (1) maintained the dogs used in his experiments on a diet composed of horse meat, a carbohydrate (rice starch) and sodium chloride. This ration is low in calcium certainly, but it is also deficient in fat-soluble A, water-soluble B, and probably in the antiscorbutic substance as well. Moreover, the protein is not of the best quality. It will be seen that, even if there were no dispute as to whether the disease which was produced in puppies by this diet was rickets or not, it would be impossible to say that the deficiency in calcium was the cause.

The gross changes in the skeleton and the alterations in the finer structure of the bones of the rats placed on the various faulty rations described, which had in common a deficiency in calcium, were so similar that a single description will serve for all.

The bodies of the animals at autopsy were very much undersized and showed quite characteristic lesions. The coat was rough and the hair was irregular in length. The skulls were often very thin and easily compressible. The eyes were normal. The incisor teeth were very brittle, yellow and easy to extract. The epiphyses of the long bones, especially at the wrist and knee, were enlarged. The shafts of the bones were slender. The thoraces were much flattened from side to side, slender, and marked by deep grooves along the costochondral junctions. When the thorax was opened, the junctions of the ribs with their cartilages were enlarged and there was marked angular deformity (Fig. 1). This extreme degree of angular deformity at the junctions of the ribs and the cartilages exceeded that found in the ribs of rats on a diet already described, low in fat-soluble A and phosphorus (4). The thymus was atrophied. The spleen was frequently greatly enlarged. There was little, if any, subcutaneous or visceral fat. The gonads were atrophic. The long bones cut easily. There were, as a rule, no fractures to be found in any of the bones except the ribs, which were usually studded throughout their length with the calluses of multiple healing fractures.

Microscopic examination of the bones revealed a lesion which was striking in its uniformity. The band of cartilage (Fig. 2) which separates the epiphyseal nucleus of ossification from the shaft was much broader than in a normal bone. The cells of the proliferative zone were separated one from another by very little matrix, and the cell bodies were almost in contact. Although there was a good deal of invasion of the cartilage by blood vessels from the shaft, the columnar arrangement of the cells was well maintained throughout. Because of the increased size and the invasion of the cartilage the cell columns seemed to radiate from the lower margin of the epiphyseal nucleus like the spokes of a wheel (Fig. 3). There was, as a rule, no calcification of the proliferative zone, though occasionally a few scattered patches of calcified matrix were present to represent a provisional zone of calcification. These calcified areas were usually at the periphery of the bone. The vascular sprouts which invaded the cartilage were very fine capillary vessels and were single or multiple. Often the invasion was carried on to a considerable distance from the shaft. Occasionally the invading vessels were arranged in tufts and destroyed wide areas of cartilage before them. The invasion of the cartilage usually took place, however, along the strands of intracellular matrix (Fig. 4). Cartilage which was directly in contact with the osteoid tissue seemed to have been protected against invasion by capillaries from the shaft. Frequently large sinuses, which had thin walls, made up of endothelium and of a few strands of connective tissue, were to be found just beneath the cartilage in the metaphysis (Fig. 5). At the junction of the cartilage with the metaphysis the cartilage cells had undergone changes like those which are described as degenerative in the bones of rachitic children. The cell cytoplasm had lost its affinity for basic dyes, and in specimens stained with haematoxylin had a pale buff color which was indistinguishable from the staining reaction given by osteoid tissue. The nuclei of the cells were seen to be smaller and more pyknotic as the shaft was approached. Finally, the cartilage cell became buried in the osteoid tissue of the metaphysis, which it apparently helped to form. In other words, this so-called degeneration is in reality, as we pointed out in our description of the bones of rats on diets low in phosphorus and fat-soluble A, a metaplasia of the cartilage cells which results in their change into bone corpuscles.

The metaphysis in these bones was narrow, in contradistinction to the metaphysis in the bones of rats on a diet low in fat-soluble A and phosphate, and appeared to be in much greater degree a product of the diaphysis. The metaphysis was made up of large numbers of trabeculae of osteoid tissue, separated by blood vessels and a delicate reticular tissue containing a few elements from the bone marrow. The spongiosa (Fig. 5) was abundant, and the trabeculae of which it was composed were very numerous and slender. This thin character of the trabeculae was another point of difference between the bones of the rats on the diets low in calcium and those of rats fed on diets low in fat-soluble A and phosphorus. The trabeculae in the latter animals were much thicker, and were fewer in number. The osteoid was broad and often lamellar (Fig. 7). The borders of the osteoid tissue were sharp and clear cut; the cells were widely separated, and their nuclei were small and stained deeply. The osteoblasts which surrounded the trabeculae were flat, closely applied to the osteoid, and altogether resembled the endothelial cells of the blood vessels. In contrast, the osteoid tissue about the trabeculae of the rats on the diets deficient in calcium alone (Fig. 8) was much more ragged. The margins faded off into the surrounding tissue. The nuclei of the bone corpuscles were larger and were very close together, and the osteoblasts which surrounded the bone, instead of being flattened cells, were of low, columnar form, and were often piled up one on the other, to form two or three layers about the trabeculae. Occasionally trabeculae were seen in the rats on the diets deficient in calcium alone which showed osteoid tissue of both types. Calcification of the trabeculae in these animals was not as dense as in the normal bones. The deposits of lime salts were granular and the calcified zone usually merged gradually into the uncalcified border. The calcified cortex of the shaft was very thin, but the cortex as a whole was usually much thickened by a compensatory development of osteoid tissue (Fig. 9)

Frequently the cortex was canalized and was found in the form of a lattice work. In situations where the bone was under particular stress, e.g., at the sites of muscular attachment, or on the concave surface of the bone an enormous amount of osteoid tissue was formed between the periosteum and the original cortex (Fig. 9). This subperiosteal formation of osteoid tissue is analogous to the so-called ''rachitic periostitis.'' It is in reality an hypertrophy to compensate for the diminished strength of the tissue used in building a bone. It is probable that canalization of the cortex of the bone and its growth in the form of a lattice has the same significance.

The bone marrow in the medullary cavity appeared normal in animals on these diets, but in the growing region of the bone the haematopoietic tissue was in a large measure replaced by delicate reticulum.

Many evidences of resorption of the trabeculae were present. Bones which showed evidences of resorption invariably contained numbers of large cells, the cytoplasm of which was filled with large basophil granules. These cells, which appeared to be derived, in part at least, from small, round lymphocyte-like forms, were arranged in a definite way along the borders of the trabeculae. They were found in the region of the bone which was being hollowed out to form a marrow cavity, but were not seen in the medulla itself. They were also present in large numbers in the interstices of the bony lattice work which represented the cortex. They were not present in the metaphysis to any extent, and were not found in the centers of ossification. We cannot say what their significance may be, but it would seem as though they must have some function to perform in connection with resorption.

Changes of the nature above described were present through the entire osseous system, but the pathological picture varied in severity in different bones. Those bones which grow most rapidly showed the most marked variations from the normal. The middle tier of ribs, the femur, the radius and ulna, and the tibia and fibula, suffered most severely. It was usual to find a more complete zone of provisional calcification in the cartilages of the vertebrae, phalanges and other smaller bones, even though the cartilages of the ribs and femur were without a vestige of lime salts. The osteoid borders of the trabeculae were widest where the most rapid growth was under way, for example, in the middle tier of ribs and the nuclei of ossification at the ends of the long bones. In the latter situation appositive bone formation must be very rapid, since in the process of growth the epiphyseal cartilage has to be replaced by bone during the period when the epiphysis as a whole is enlarging to keep pace with the growth of the entire body.

Broadly speaking, these bones had certain characteristics in common with the bones of rachitic children. The cartilages were not calcified, the epiphyseal line was irregular, and there was a considerable degree of invasion of the cartilage by blood vessels from the shaft. Osteoid tissue was formed in abnormal amounts, and the failure of calcification to occur resulted in weakness of the skeleton, with deformity of the thorax, cranio-tabes, etc. Attempted compensation for the lack of calcification of the bones was seen in the over-production of preosseous tissue and the subperiosteal hypertrophy of the cortex. On the other hand, the changes in the bones which were produced by the above described diets differed from rickets in several important particulars. The cartilage cells retained their columnar arrangement in spite of a considerable degree of invasion by blood vessels. Moreover, as Schmorl (7) has pointed out in the bones of puppies fed on low calcium diets, these bones showed evidences of a much greater degree of resorption than is usually seen in the bones of rachitic children.

If rats which had been confined for some weeks to one of the diets described above were given cod liver oil for a few days before they were killed, a healing process was induced which was evidenced by the formation of a provisional zone of calcification in the proliferative cartilage of the bones. If the animal was allowed to live for a sufficient length of time after the administration of cod liver oil was begun, the rachitic process in the bone was healed, and instead a condition of osteoporosis developed. If cod liver oil was included in the dietary formula from the beginning of its administration, the animals were never normal, and the bones at autopsy were absolutely different from those of rachitic rats. They showed a peculiar pathological condition which will be fully discussed in a subsequent paper.

When adequate amounts of calcium carbonate were added to the diets, the condition which developed in the skeleton was normal.

CONCLUSIONS.

1. Rats which have been fed on the diets described above, which are low in calcium, but which contain a sufficient supply of the fat-soluble A and have an approximately normal content of phosphate,

develop a pathological condition of the bone which has certain fundamental resemblances to human rickets. The condition is characterized by increased persistence of the epiphyseal cartilage, its invasion by blood vessels from the shaft, failure in lime salt deposition, the formation of a mixed zone between the cartilage and shaft (the rachitic metaphysis), and the over-production of osteoid tissue. Gross deformities which cannot be differentiated from those caused by rickets are seen at autopsy.

2. The above described pathological condition differs from rickets, as that disease commonly occurs in man, in that the arrangement of the proliferative zone of cartilage cells is maintained, and that the evidences of bone resorption in the diaphysis are excessive.

3. The rickets-like condition of the skeleton does not develop, if the deficiency in calcium is compensated for by the addition to the diet of calcium carbonate.

4. The rickets-like condition never develops, if the animal is allowed to receive cod liver oil, and the administration of cod liver oil, when the condition is well advanced, is followed by healing of the lesion with the formation of an osteoporotic bone.

5. Since this pathological condition in the skeleton is in no way prevented by an amount of butter fat which is amply sufficient to protect the animals against keratomalacia, it is necessary to assume that butter fat exercises a very feeble influence in protecting animals against the development of rickets-like lesions.

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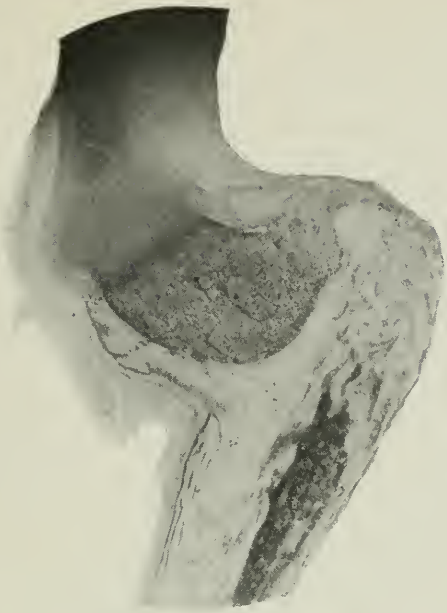

Fig. 1.

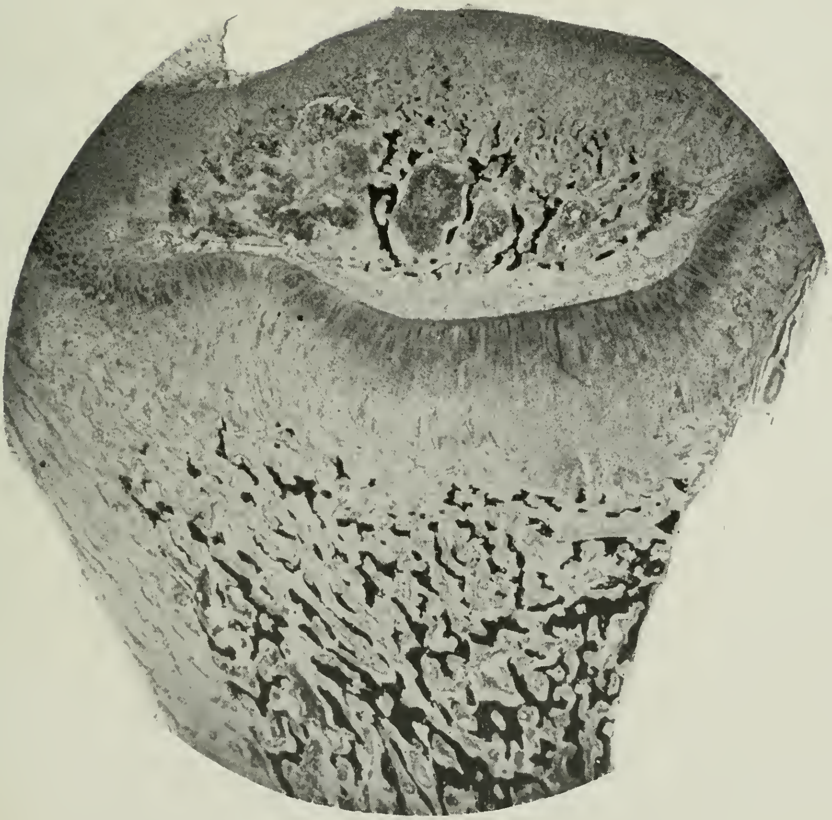

Fig. 2.

PLATE XXV.

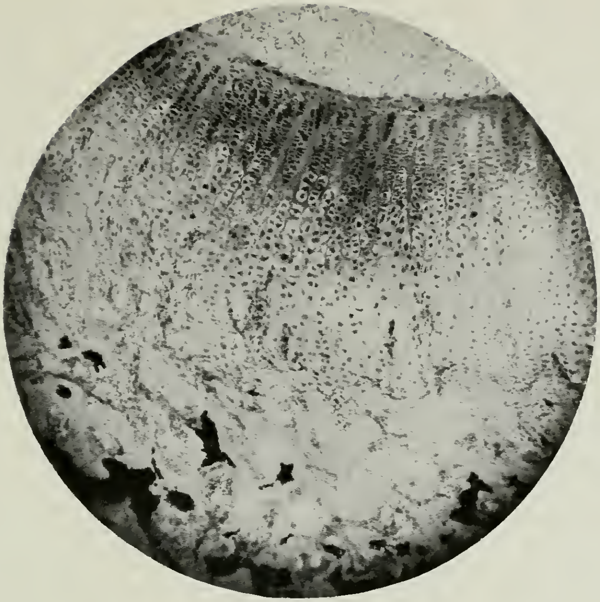

Fig. 3.

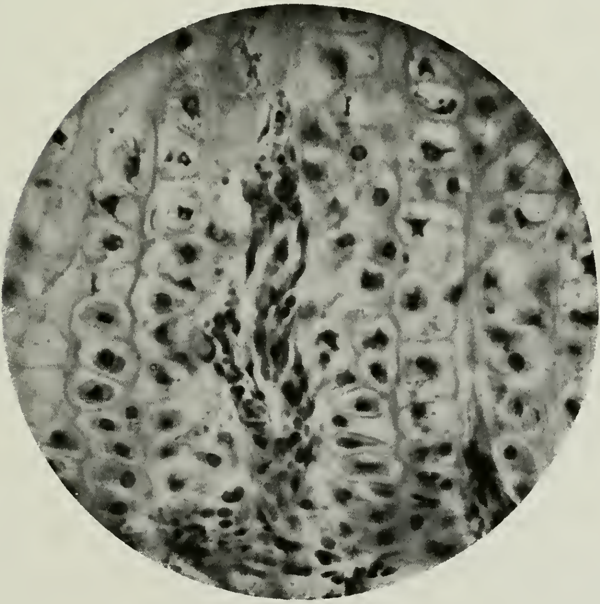

Fig. 4.

PLATE XXVI.

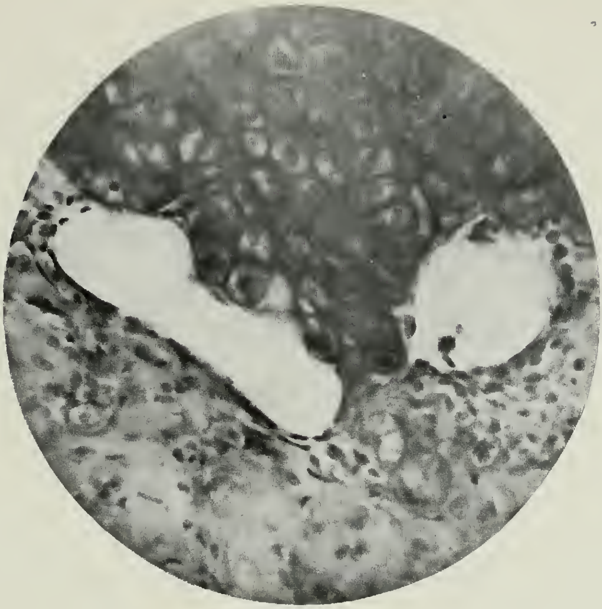

Fig. 5.

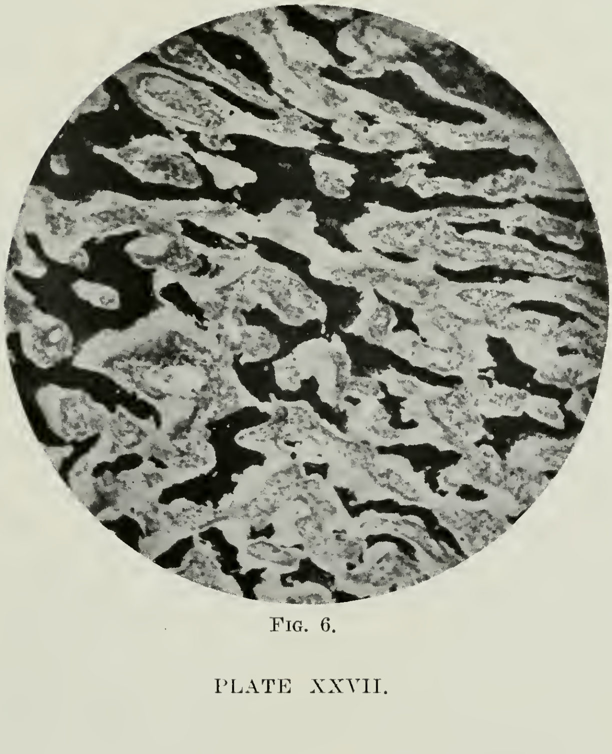

Fig. 6.

PLATE XXVII.

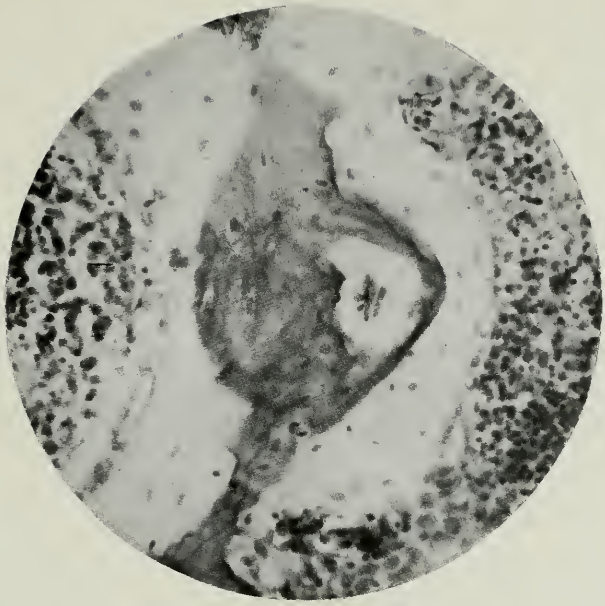

FIG. 7.

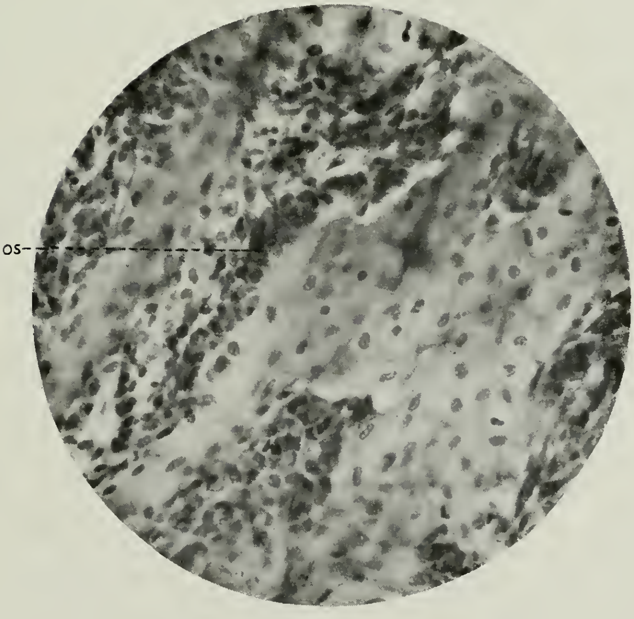

FIG. 8.

PLATE XXVIII.

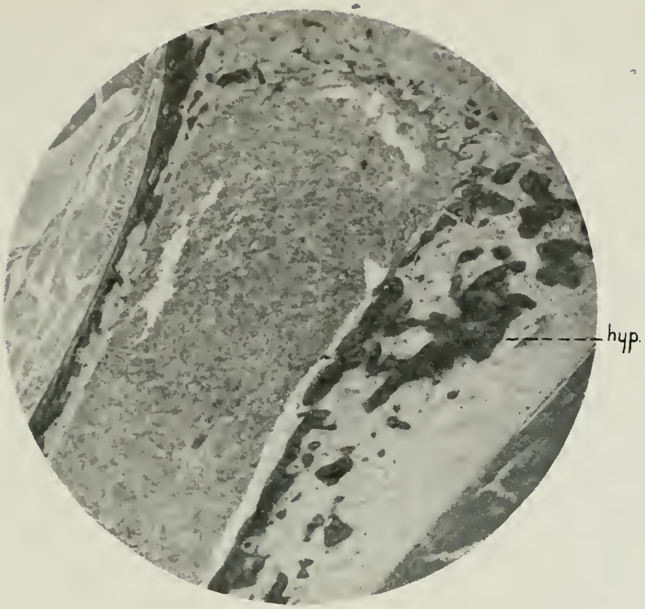

Fig. 9.

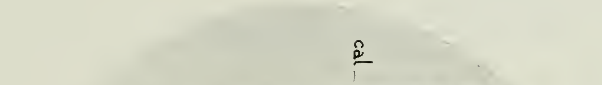

Fig. 10.

PLATE XXIX.

EXPLANATION OF PLATES.

PLATES XXV.

FIG. 1. Longitudinal section through the cartilage and shaft of the tenth rib of a rat on a diet low in calcium, to show the extreme angulation at the costochondral junction. (Leitz micro-summar objective 135 mm.)

FIG. 2. Photomicrograph of a longitudinal section through the proximal end of the tibia of a rat on the diet of lot 2810. (Same magnification as in figure 1.)

PLATES XXVI.

FIG. 3. This photograph shows the invasion of the lower borders of the epiphyseal cartilage by capillary vessels from the diaphysis, and the radial arrangement of the columns of cartilage cells. Note the small amount of intercellular matrix and the close apposition of the cells. (Leitz objective No. 3.)

FIG. 4. Photograph with Leitz objective No. 6 showing a capillary blood vessel invading the epiphyseal cartilage. The vascular sprout is forcing its way along the septum which separates one cartilage cell from another.

PLATES XXVII.

FIG. 5. Large blood sinuses between the epiphyseal cartilage and the shaft of the bone. (Same magnification as figure 4.)

FIG. 6. Spongiosa of the shaft of the femur from a rat on diet of Lot 2581. Note the numerous, slender trabeculae, each surrounded by a zone of osteoid tissue. (Leitz No. 3 objective.)

PLATES XXVIII.

FIG. 7. High power photomicrograph of a trabecula with appositive osteoid. The cells in the osteoid are widely separated, the nuclei of the greater number are small. The osteoid border is wide and its edges are sharply cut. In the calcified portion of the trabecula is seen the cross section of a perforating blood vessel.

FIG. 8. High power photomicrograph (Leitz objective No. 6) to show the resorptive osteoid from the bone of an animal on a diet low in calcium, No. 2581. The osteoid border of these trabeculae is narrow. Calcification of the trabeculae is very poor. The trabeculae are crowded with large bone corpuscles, and at the border of the trabecula is seen a mass of osteoblasts four or five cells in depth. (O. S.)

PLATES XXIX.

FIG. 9. Longitudinal section through shaft of long bone to show subperiosteal-hyperplasia (Hyp.).

FIG. 10. Photomicrograph of callus about a spontaneous green stick fracture (f.). The (cal.), which is separated from the cortex by a mass of bone marrow, is almost entirely composed of osteoid tissue.

STUDIES ON EXPERIMENTAL RICKETS.

VII. THE RELATIVE EFFECTIVENESS OF COD LIVER OIL AS CONTRASTED WITH BUTTER FAT FOR PROTECTING THE BODY AGAINST INSUFFICIENT CALCIUM IN THE PRESENCE OF A NORMAL PHOSPHORUS SUPPLY.

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PLATES XXX TO XXXIII. (FIGURES 1 TO 6.)

In the first paper of this series* we pointed out that a diet which consisted of cereal grains, legume seeds, and muscle meat, supplemented with sodium chloride and sodium bicarbonate, was capable of supporting approximately normal growth, and the animals were capable of producing and rearing some young when 3 per cent. of cod liver oil was added to it. Essentially the same mixture, supplemented with 5 per cent. of butter fat, instead of the cod liver oil, failed to induce appreciable growth and led to permanent stunting, early deterioration and death. Our earlier studies showed that cereal, legume seed and muscle meat mixtures require additions of calcium, sodium, chlorine, and fat-soluble A, to render them sufficiently complete to make possible normal growth to maturity and approximately normal fertility.

The striking point about the experiments referred to is that the animals were so much more effectively protected against injury, due to the low calcium content of the diet by cod liver oil, than by butter fat, even when much more of the latter than of the former was added to the diet. This suggests that either (*a*) cod liver oil contains a dietary essential in abundance which is contained but sparingly in butter fat, or (*b*) that at least two organic nutritive principles are associated with certain fats, and that both butter fat and cod liver oil contain each, but in proportions widely different. With the following diet

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* Jour. Biol. Chem., 1921, xlv, 333.

	Per cent.
Rolled oats	30.0
Casein	18.0
Salts (185)	3.7
Agar-Agar	2.0
Dextrin	46.3

we have repeatedly demonstrated that xerophthalmia, but never rickets, develops. This we have discussed more extensively elsewhere.* The bones are *osteoporotic* but not *rachitic*. The inorganic constituents are present in a proportion which makes calcification complete, but some substance necessary for the formation of normal osseous tissue is lacking. Either butter fat or cod liver oil will furnish the necessary constituent in this particular diet.

With the following diet

No. 3041.

	Per cent.
Wheat	30.0
Corn	20.0
Peas	10.0
Navy beans	10.0
Rolled oats	10.0
Polished Rice	10.0
Casein	10.0

xerophthalmia never develops, but a pathological condition bearing fundamental resemblances to rickets invariably does. In this diet the faults are deficiency in calcium and low fat-soluble A. It should be kept in mind that in this diet fat-soluble A is not absent. Perhaps an estimate that it furnishes 50 per cent. of the optimal requirement of the growing rat for this substance would not be far from correct.

We have abundantly demonstrated, however, that the addition of butter fat alone to this diet will not cause optimal growth and skeletal development. It will, as a matter of fact, judging from the growth curves so far obtained, improve the condition of animals on this diet scarcely at all, even though the amount added may be anywhere between 3 and 20 per cent. of the food mixture. This observation we can now bring into contrast with the results of feeding a similar food mixture in which the deficiency was calcium alone, and on which the condition of the animals differed in the most pronounced degree, depending on whether the fat added to the diet was cod liver oil or butter fat.

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* Johns Hopkins Hospital Bulletin, May, 1921.

Calcium is a more serious limiting factor as regards growth in a seed and steak diet than is any organic substance. The presence of even liberal amounts of butter fat (ten to fifteen times the amount necessary to protect against ophthalmia on a diet of isolated food substances) proves to be little or no benefit in preventing injury due to insufficient calcium. Cod liver oil, however, is of much higher biological value for protecting rats against a lime salt deficiency. This protection is manifested in stimulation of growth and in greatly improved appearance, but although these rats may seem exceptionally well-nourished and appear to possess great vigor, they are actually in a state of nutritional instability. As a matter of fact, their vitality is far below that of rats on highly satisfactory diets. Their weakness, moreover, is shown in a spectacular way in its effect on the females as regards nursing their young. Such female rats show characteristic behavior, they are nervous and apprehensive, in walking they stand with legs more extended, and with the body held higher than is the case with a normal animal. Their gait is unsteady and slightly tottering. When at rest they have some difficulty in getting on their feet. Not infrequently the mothers die after nursing litters for 16–18 days, although they appear externally to be in good condition. This is especially to be emphasized in view of the fact that the animals on these low calcium diets which were enabled to grow fairly well by reason of the administration of cod liver oil were distinctly below the normal in their physical development.

They sometimes succeed in weaning their young, they frequently do not, especially with second or later litters. They only rarely destroy their young soon after birth, but are not able to nourish them satisfactorily with their milk. The difference between the protective value of butter fat and cod liver oil, however, is manifested in the difference of fertility of rats fed on one of these fats, as contrasted with the other. We have never been able to secure young with diets low in calcium of the type described in Chart 1. On the other hand, cod liver oil to the extent of 1 to 3 per cent. is very effective in promoting fertility. These young restricted to the mother's diet are short and stocky, rough-haired, with very poor skeletons, but little calcified, and die early.

Butter fat, although a good source of fat-soluble A (anti-ophthalmic substance) does not in the absence of added calcium prove effective as an agent in the calcification of bone, but in diets markedly deficient in calcium with an approximately normal content of phosphorus, cod liver oil in surprising contrast to even much larger amounts of butter fat serves to protect the animal against defective calcification of the osseous tissue. The bones of rats which have been raised on a diet in which butter fat supplies the only source of the fat-soluble organic factor show a severe rickets-like condition which we have described at length elsewhere. These bones contain much osteoid tissue. The cartilages are not calcified and a pathological metaphysis is formed (Fig. 3). These animals show gross skeletal changes which can not be differentiated from those which are the result of rickets.

Rats of the second generation on such diets (Lot No. 2733) have bones which differ almost unbelievably from those of the parents. The cartilages are somewhat broader than normal, and very irregularly, or not at all calcified. There is very little invasion of the cartilage, however, and very little matrix between the cartilage cells. The bone itself is a mass of very slender trabeculae which may be fairly well calcified or may be composed almost entirely of osteoid tissue. In either event the trabeculae are filled with enormous bone corpuscles with large nuclei. Many of the bone corpuscles are surrounded by a clear area, although they are in touch with the surrounding bone through processes of their cytoplasm. The osteoblasts which surround the slender trabeculae are of enormous size and of low columnar or polyhedral form. Often there are several layers of them about a single trabecula. Sometimes the osteoblasts appear to be separated from the trabeculae which they surround, by capillary blood vessels. At least blood cells are to be found between the trabecula and the osteoblasts which surround it, though the vessel wall is usually not visible. In gross these animals are distinguished by the extreme slenderness of their bones. The skeleton is very fragile, fractures of the long bones are uncommon, but there are always multiple fractures of the ribs. More than 100 fractures have been present in a single thorax. The callus which is formed during the healing process is very scanty. Neither the epiphyses of the long bones nor the costochondral junctions are at all enlarged, and there is no gross deformity of the chest wall. We shall discuss the pathology of these animals and its significance in more detail in another communication.

We have, therefore, been fortunate in establishing experimental

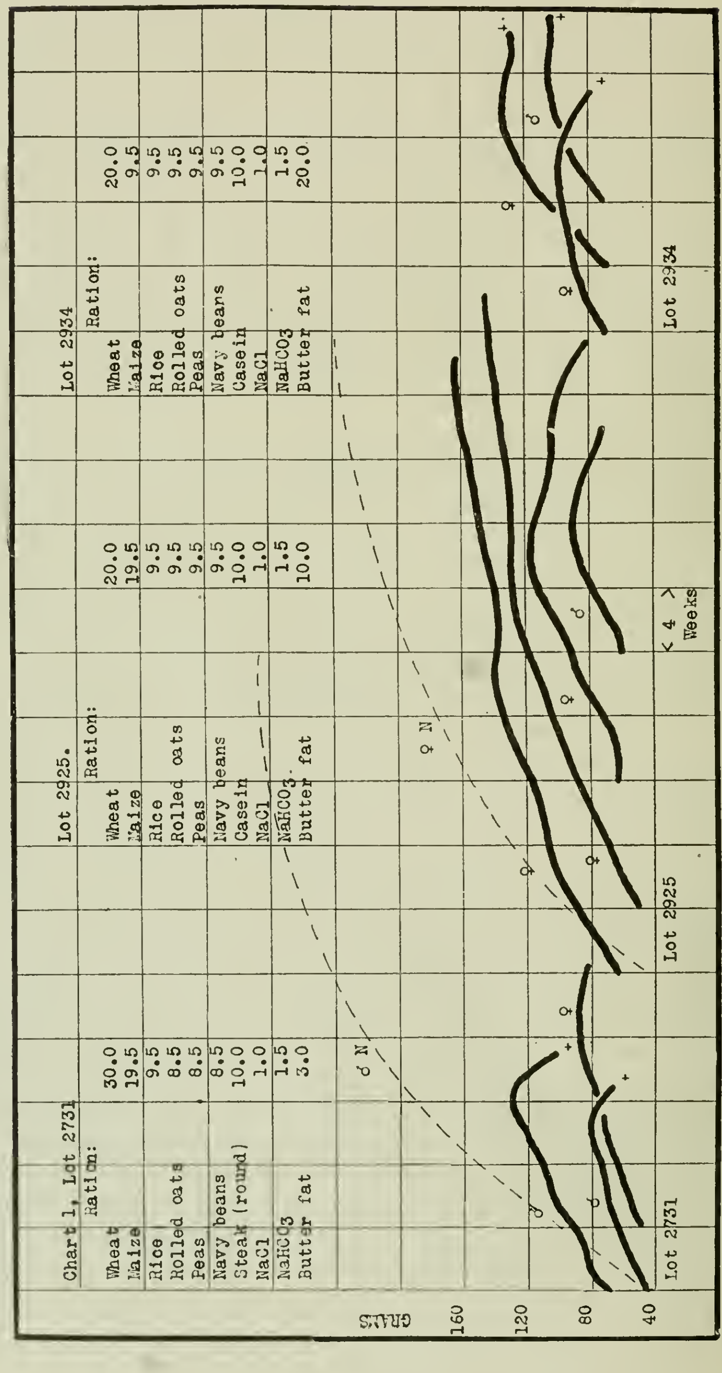

Chart 1, Lot 2731

Ration:

Wheat	30.0
Maize	19.5
Rice	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Steak (round)	10.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	3.0

Lot 2925.

Ration:

Wheat	20.0
Maize	19.5
Rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Casein	10.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	10.0

Lot 2934

Ration:

Wheat	20.0
Maize	9.5
Rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Casein	10.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	20.0

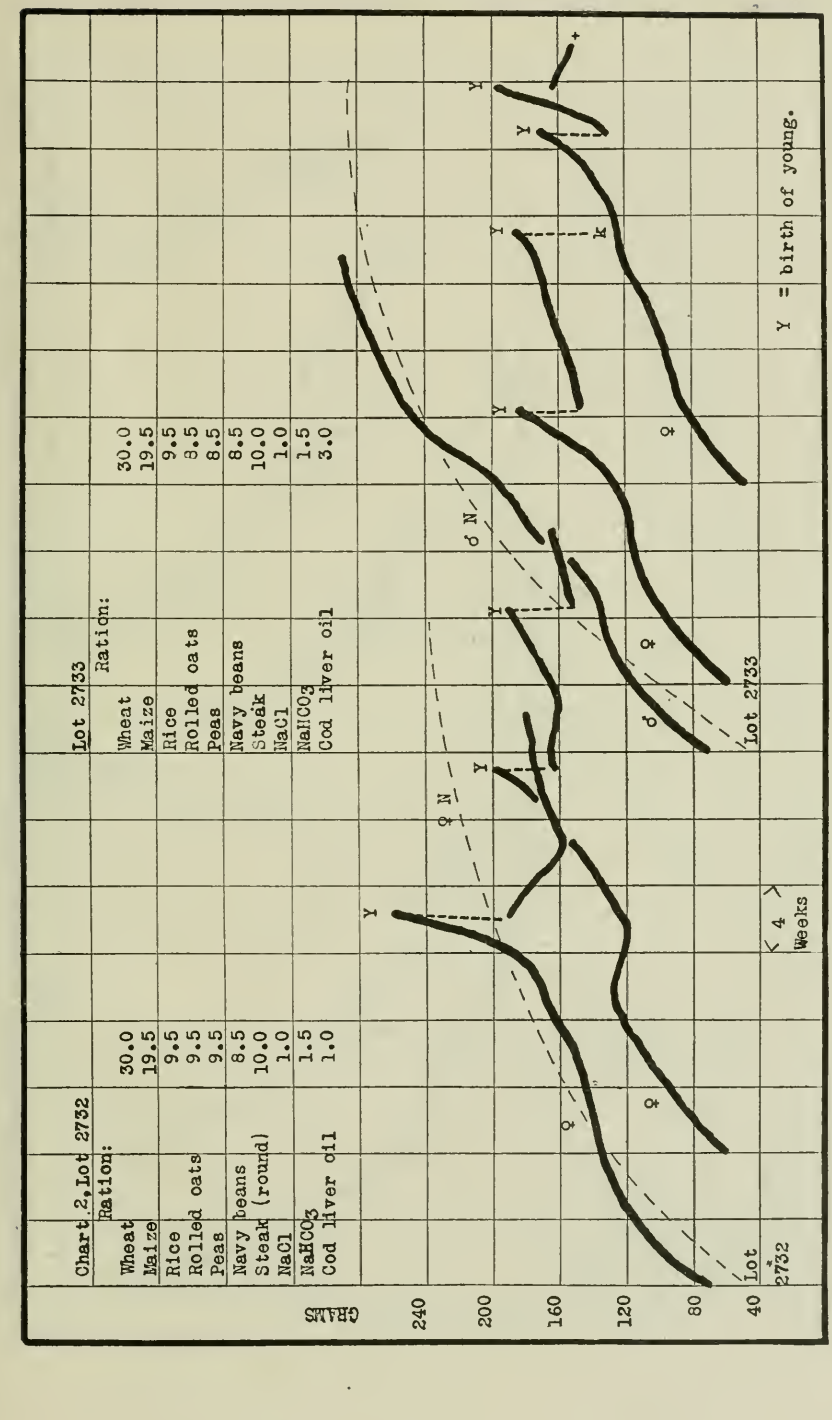
Chart 2, Lot 2732
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 9.5
Peas 9.5
Navy beans 8.5
Steak (round) 10.0
NaCl 1.0
NaHCO3 1.5
Cod liver oil 1.0
Lot 2733
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 8.5
Peas 8.5
Navy beans 8.5
Steak 10.0
NaCl 1.0
NaHCO3 1.5
Cod liver oil 3.0
GRAMS
240
200
160
120
80
40
Y
Y
Y
Y
Y
Y
Y
♀
♀
♀ N
♂ N
♀
♂
♀
Lot 2732
Lot 2733
< 4 > Weeks
Y = birth of young.

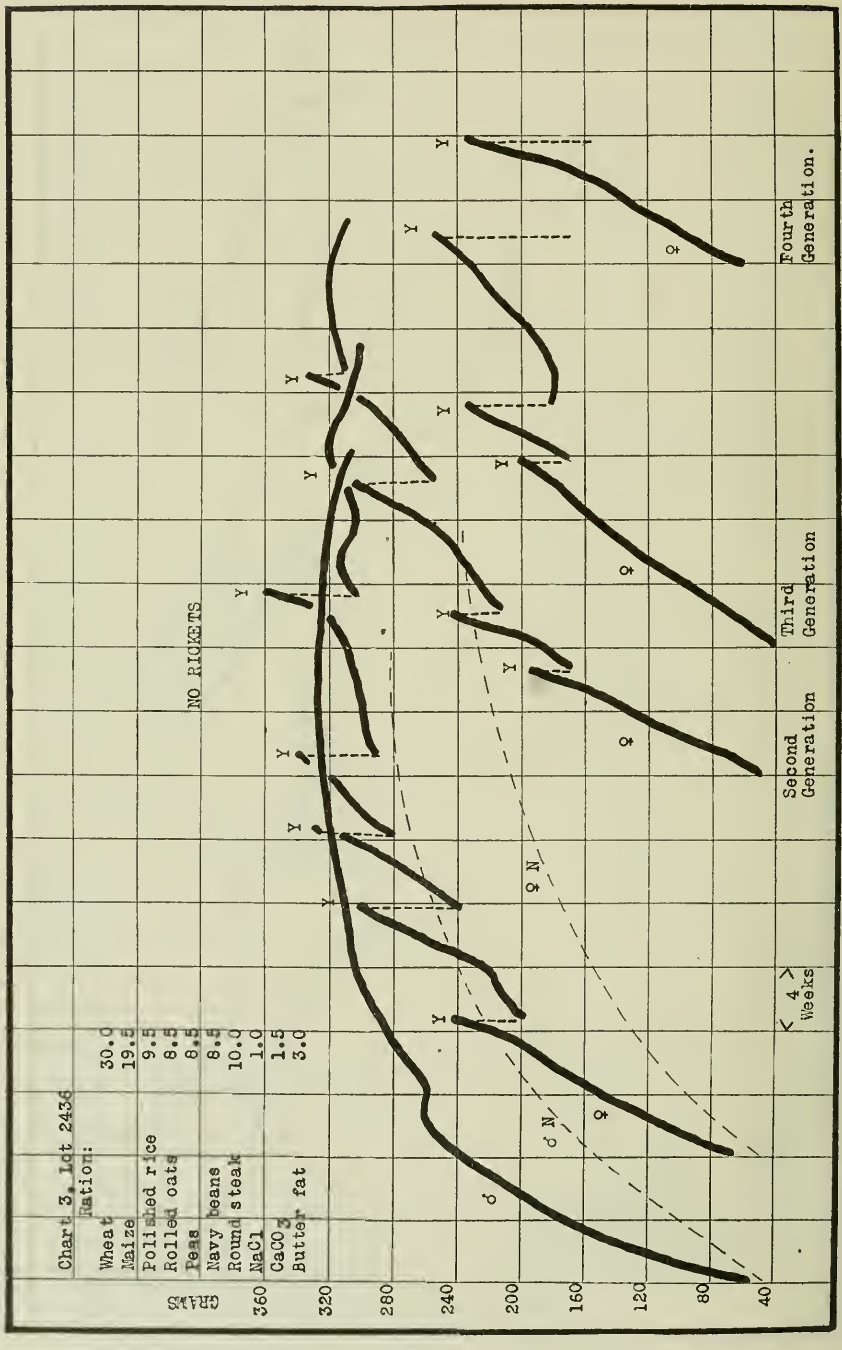
Chart 3, Lot 2436
Ration:
Wheat 30.0
Maize 19.5
Polished rice 9.5
Rolled oats 8.5
Peas 8.5
Navy beans 8.5
Round steak 10.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
GRAMS
360
320
280
240
200
160
120
80
40
NO RICKETS
Y
♂
♀
♂ N
♀ N
< 4 > Weeks
Second Generation
Third Generation
Fourth Generation.

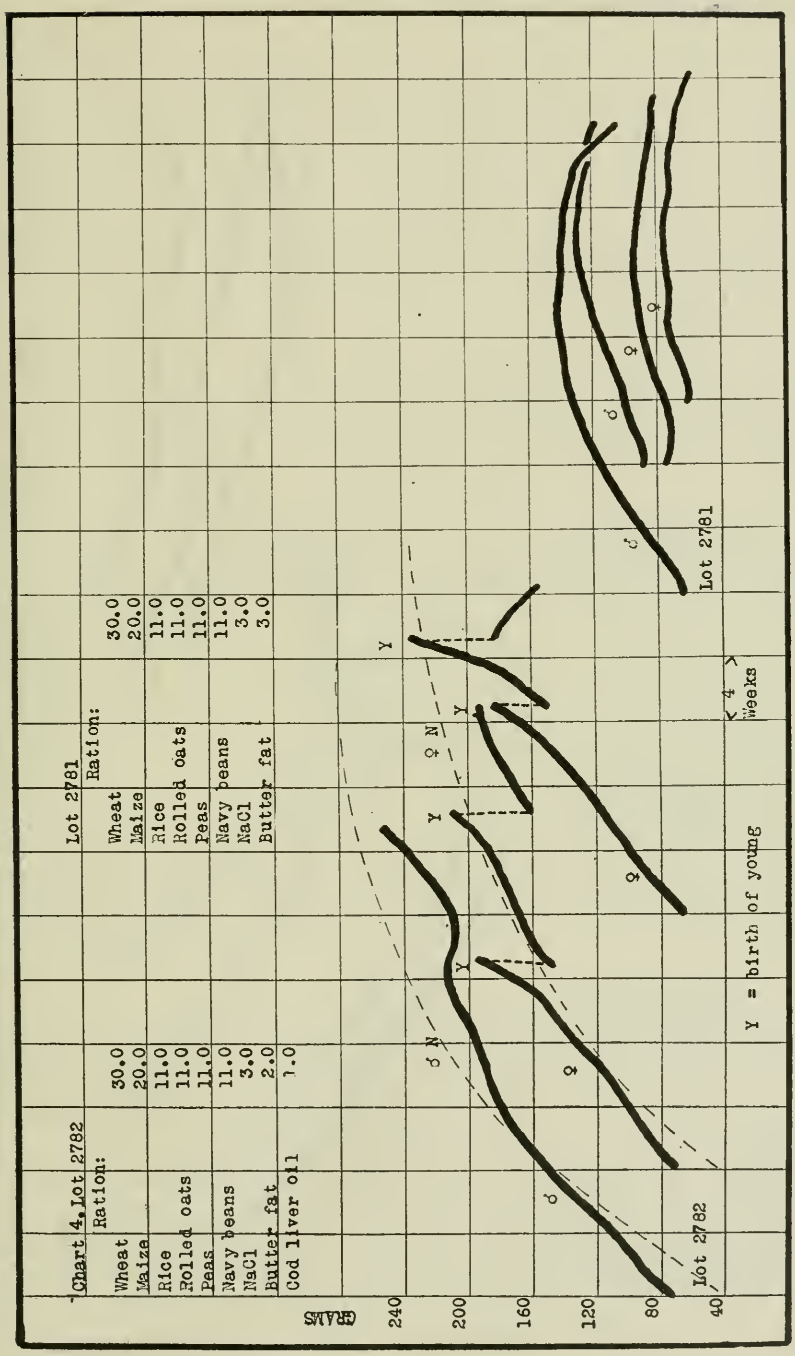
Chart 4. Lot 2782
Ration:
Wheat 30.0
Maize 20.0
Rice 11.0
Rolled oats 11.0
Peas 11.0
Navy beans 11.0
NaCl 3.0
Butter fat 2.0
Cod liver oil 1.0
Lot 2781
Ration:
Wheat 30.0
Maize 20.0
Rice 11.0
Rolled oats 11.0
Peas 11.0
Navy beans 11.0
NaCl 3.0
Butter fat 3.0
GRAMS
240
200
160
120
80
40
♂ N
♀ N
♂
♀
Y
Lot 2782
Lot 2781
Y = birth of young
< 4 > Weeks

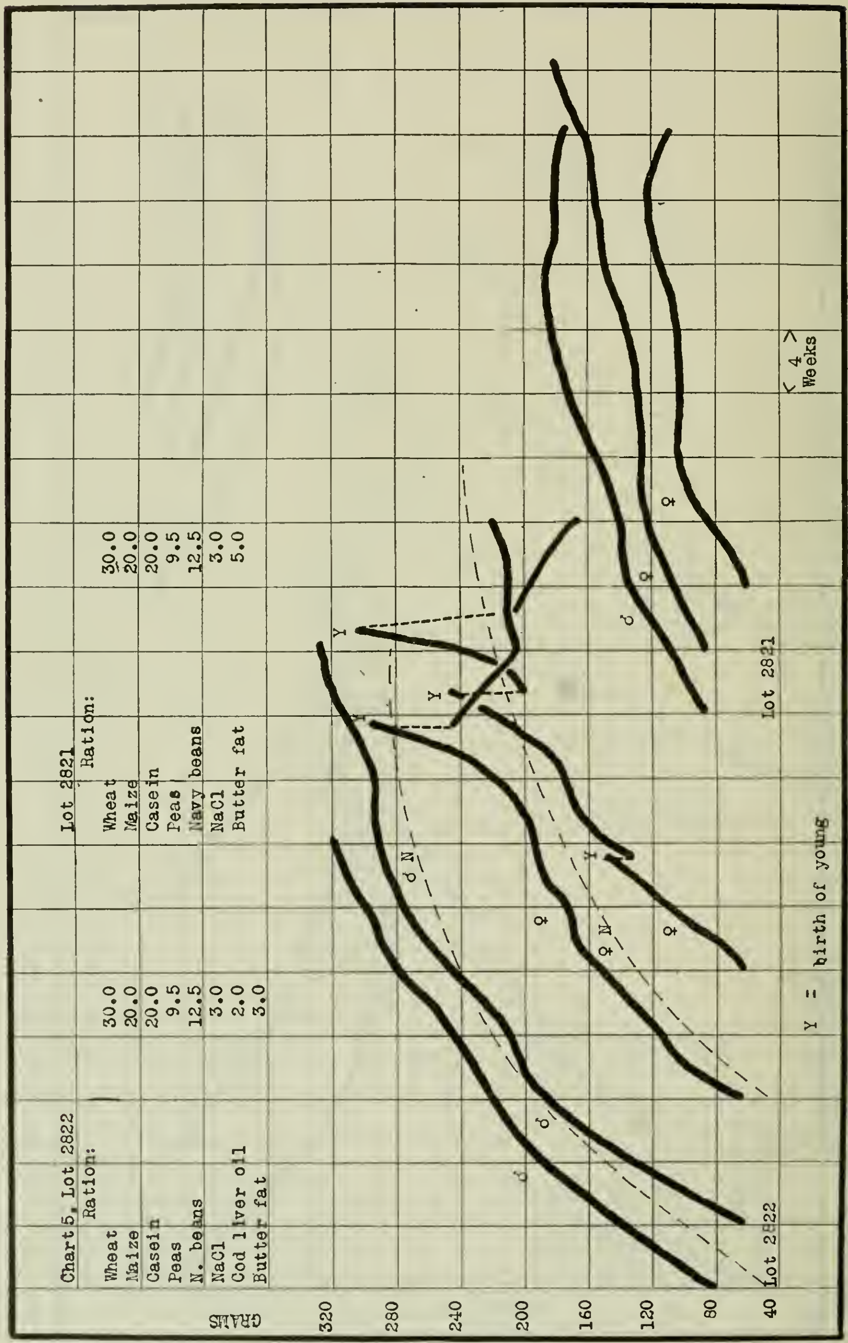

Chart 5, Lot 2822
Ration:

Wheat	30.0
Maize	20.0
Casein	20.0
Peas	9.5
N. beans	12.5
NaCl	3.0
Cod liver oil	2.0
Butter fat	3.0

Lot 2821
Ration:

Wheat	30.0
Maize	20.0
Casein	20.0
Peas	9.5
Navy beans	12.5
NaCl	3.0
Butter fat	5.0

conditions which clearly show that butter fat and cod liver oil not only contain the anti-ophthalmic substance, fat-soluble A, but that cod liver oil even in small amounts (2–3 per cent. of the diet) will, under the experimental conditions here described, protect against the baneful influence of serious grades of calcium starvation when other factors in the diet are satisfactory, whereas butter fat does not possess this power in a degree demonstrable by our method of study. It is probable that butter fat also contains minimal amounts of this organic factor, which is so abundant in cod liver oil, but the antirachitic effect of any samples of butter fat which we have employed is certainly very small as compared with the antixerophthalmic potency. This difference between the effects of cod liver oil, as contrasted with butter fat, can up to the present time be demonstrated only under the experimental conditions here described, viz., where there is a decided deficiency in the calcium content of the food.

In all the reproduction records which we have observed in these experiments, in which cod liver oil in the absence of sufficient calcium in the diet stimulated growth and improved the vitality of the animals so as to enable them to produce young, we have never seen a case where the mother destroyed her young even though she could not nourish them satisfactorily with her milk. In a recent discussion of the abnormal attitude which mother rats frequently take toward their young when restricted to diets in which the protein is inadequate, we called attention to the infanticidal tendency caused by this deficiency. It is interesting that this tendency to destroy the young is not caused by any fault in the diet which places the mother in a condition of nutritive instability in which she may easily break down while nursing a litter, or in which she fails in her attempt to induce normal nutrition in her offspring.

Conclusions.

1. Diets in which the calcium is deficient, but in which the phosphate content is near the optimum, cause injury to young rats, interfering with growth and causing characteristic disturbances in the growth and ossification of the skeleton.

2. Butter fat, even in liberal amounts, fails to be very effective in protecting the animals against this injury due to calcium deficiency.

3. Cod liver oil, on the other hand, is remarkably effective in promoting growth under the experimental conditions described, and in protecting young animals against the harmful effects of partial calcium starvation.

4. These results suggest the possibility that a dietary essential distinct from the anti-ophthalmic substance (fat-soluble A) may exist. If this is the case this would appear to be present in butter fat in small amounts, but to be very abundant in cod liver oil.

5. When the amount of calcium in the diet is below the optimum, but the phosphorus is normal, the rôle of the antirachitic organic substance is to direct as far as possible the bone development in the direction of normal.

6. When the calcium and phosphorus content of the diet, and presumably the other mineral elements, are nearly optimal, the amount of antirachitic substance needed by the body is small, and its function appears to be regulatory. The need for this substance is greatly increased when there is partial starvation for calcium.

7. We have not found it possible to demonstrate experimentally a difference between the effects of cod liver oil and butter fat when the content of the diet in calcium and phosphorus is near the optimum, the other elements in the diet being satisfactory.

EXPLANATION OF CHARTS.

Chart 1. Lots 2731, 2925 and 2934 illustrate the failure of butter fat, when added to the extent of 3 to 10 and 20 per cent. respectively, to stimulate growth in animals fed the diets described in the chart. The sole deficiency of appreciable magnitude in these food mixtures is calcium. This is shown by a comparison of the curves of these groups with those of Lot 2436, Chart 3. In the latter group the diet was identical with that of 2731, except that the sodium bicarbonate was replaced by calcium carbonate. The addition of calcium caused normal growth, high fertility and low infant mortality. Even a fourth generation female successfully reared young of the fifth generation on this diet, and there was no noticeable deterioration in the vitality of these descendants.

Lots 2731, 2925 and 2934 were in a ''twilight zone'' of nutritional instability. There appears to be no significance in the somewhat longer life span of some of Lot 2925 as compared with the other groups.

Even 3 per cent. or less of butter-fat contains sufficient fat-soluble A to prevent ophthalmia, and is sufficiently potent, so that when both calcium and phosphate are present in optimal concentrations at least fairly satisfactory bone growth is possible. Even 20 per cent. of butter fat does not, however, further appreciably promote their well-being under the conditions of those experiments where the calcium and phosphorus content of the diet are unsatisfactory.

The interpretation of experimental data such as we are describing, with a view to determining whether there are two uncharacterized dietary factors in such fats as butter fat or cod liver oil, in which hitherto only fat-soluble A was recognized, is rendered very difficult because of the impossibility up to the present time of separating the two, if there be two, and studying them independently. We are confronted with the question as to whether, when all indispensable organic and inorganic substances are contained in a food, bone formation can take place in a fairly normal manner, without the aid of the substance designated above as X. It is difficult to explain the observed results on any other assumption. It seems very suggestive, that as the content of calcium and of phosphate in the food deviates from the optimum ratio, either upward or downward and in either direction, there arises a need for a regulatory substance which aids in directing the osteoblastic activity toward the most favorable objective from the standpoint of the individual. Its purpose is to turn the bone corpuscle toward normality. The need for this hypothetical X becomes greater, the more decided the departure of the diet from the optimum in calcium and phosphorus ratios. When these components are present in optimal amounts the need for the regulatory factor certainly is very small as compared with what it is when either calcium or phosphate or both are furnished by the diet in abnormal amounts or proportions.

Chart 2. Lots 2732 and 2733 were restricted throughout the experiment to diets which were identical with that of Lot 2731 (Chart 1), except that cod liver oil replaced the butter fat. The remarkable benefit which the animals derived from even such small amounts of cod liver oil as 1 to 3 per cent., as contrasted with even 10 or 20 per cent. of butter fat, is easily seen by comparing the curves of Chart 2 with those of Chart 1. The former not only grew much better, but looked well-nourished and produced and reared young to the weaning stage, whereas the animals whose records are shown in Chart 1 were rough-haired and poorly nourished, and were infertile.

Lot 2732 contained 4 females. Three of these had four litters (18 young). These were never normal, but the mothers reacted normally toward them and never destroyed them. The young remained under-sized and developed badly fractured ribs. Their abdomens were distended and they died early.

Lot 2733 had a similar history so far as reproduction was concerned.

Chart 3. Lot 2436 shows in a striking manner the effect of adding 1.5 per cent. of calcium carbonate to the diet of Lot 2731, Chart 1. These two groups of curves should be compared with Lots 2732 and 2733, Chart 2, and with Lots 2925 and 2934, Chart 1. The amount of calcium contained in a seed and steak mixture is very inadequate to meet the needs of the growing rat for this element. The small content of butter fat (3 per cent.) sufficed in this case, where the content of both calcium and phosphorus were near the optimum, to meet all the needs of the osseous system for any organic factor which is concerned with the functioning of the osteoblast. Yet in the absence of sufficient calcium in a nutritive situation otherwise identical, 20 per cent. of butter fat failed signally as compared with 1 or 3 per cent. of cod liver oil, to preserve the health and vitality of the animals or to enable them to grow. Such evidence may possibly suggest the supposition that cod liver oil contains a substance distinct from fat-soluble A and not hitherto recognized in working hypotheses relating to the interpretation of the causes of nutritive failure. In this event butter fat would have to contain traces of this substance, for when the calcium and phosphorus are present in favorable ratios and in near optimal concentrations, as in the diet of Lot 2436, butter fat furnishes everything necessary for so completing the seed-steak formula used as to make possible the formation of normal bones.

Chart 4. Lots 2782 and 2781 were fed diets essentially similar to Lots 2822 and 2821, Chart 5, except that the phosphorus content was lower by about .11 gram per 100 grams of food mixture. This was due to the substitution of polished rice and rolled oats for 20 per cent. of casein. The calcium content of the food was, like that of the other seed-steak or seed-casein mixtures described in this paper, decidedly below the minimum on which growth can take place in the absence of the protective action of cod liver oil.

These two groups of rats serve to illustrate again the great superiority of cod liver oil over butter fat in *the absence of added calcium,* in promoting growth and reproduction and in raising their vitality. We are inclined to hold the view, provisionally, that this is explained on the hypothesis that cod liver oil contains a regulating substance which exerts a profound influence on the behavior of the bone corpuscles and perhaps on other tissues of the body as well.

Chart 5. Lots 2822 and 2821 were fed diets which differ from those of Lots 2782 and 2781, Chart 4, essentially in containing about .12 gram more phosphorus per 100 grams of ration, owing to the substitution of casein for rice and rolled oats. A comparison of Lots 2822 with 2782 shows clearly the value of the additional phosphorus in the former. The protein factor was of very minor importance, for we have seen male rats reach weights exceeding 400 grams on the same proteins as are furnished by the ration of Lot 2782, when adequately supplemented with respect to all other factors.

These two groups of animals serve to illustrate further the great superiority of cod liver oil over butter fat in *the absence of sufficient calcium.* We have elsewhere in this paper discussed the probable explanation for this.

EXPLANATION OF PLATES.

Plate XXX.

Fig. 1. This photograph shows a rat which was representative of Lot 2821 (Chart 5). These were restricted from an early age to a diet consisting of wheat, maize, peas, navy beans, casein, NaCl and butter fat. The formula of the food mixture is given in Chart 5. The main deficiency in this food mixture is lack of calcium, which was pronounced, but not extreme. 100 grams of this food contained 0.398 gram of calcium. The optimum calcium content is about 1,000 gram. There was an unfavorable ratio between the calcium and phosphorus in this diet. This was sufficiently marked to cause abnormalities in the bones, somewhat suggesting rickets.

The animals shown in Figs. 1 and 2 illustrate the effect of cod liver oil as contrasted with butter fat in protecting the body against partial calcium starvation, and the harmful effect of unfavorable ratios between calcium and phosphate. The rat shown in Fig. 1 had an abundance of fat-soluble A for the promotion of growth when the inorganic components of the diet are satisfactorily constituted, and to prevent the development of ophthalmia. It is far less effective than cod liver oil, however, for regulating the development of the bones when the diet is deficient in the manner here described.

The animals fed the diet of Lot 2821 were far less well developed physically and were infertile. The same food mixture containing cod liver oil induced good

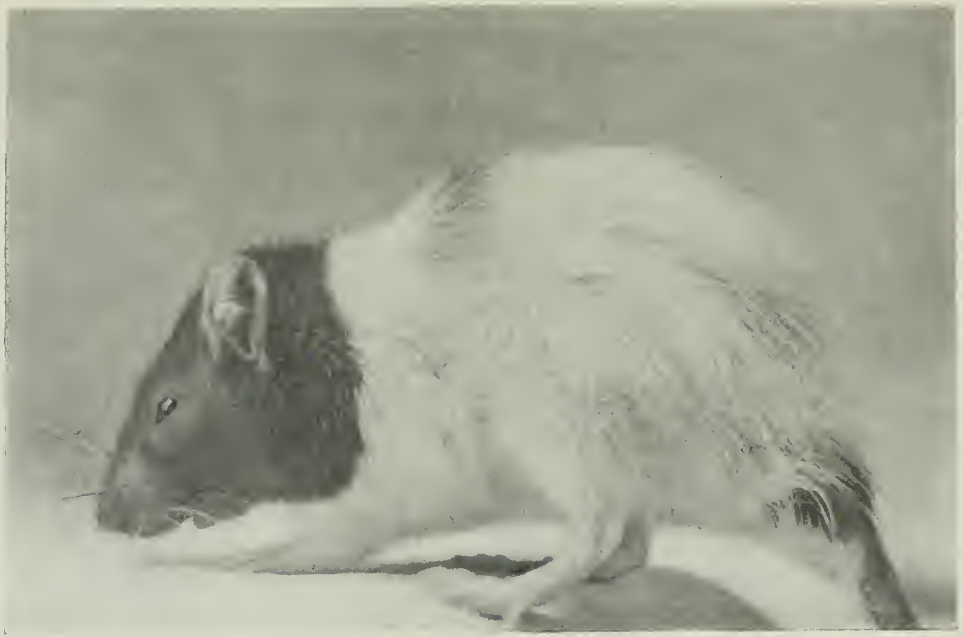

Fig. 1.

PLATE XXX.

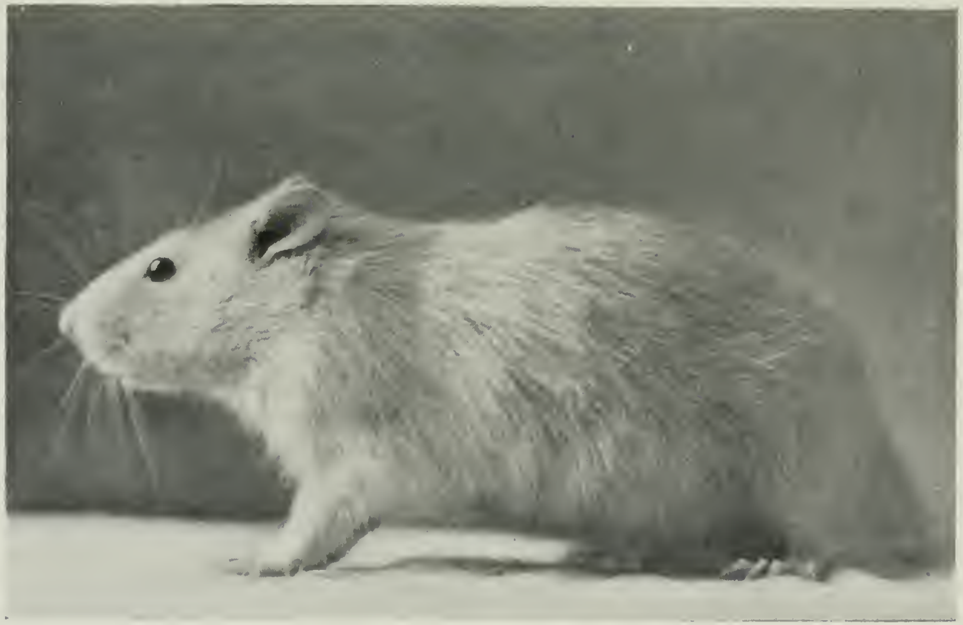

Fig. 2.

PLATE XXXI.

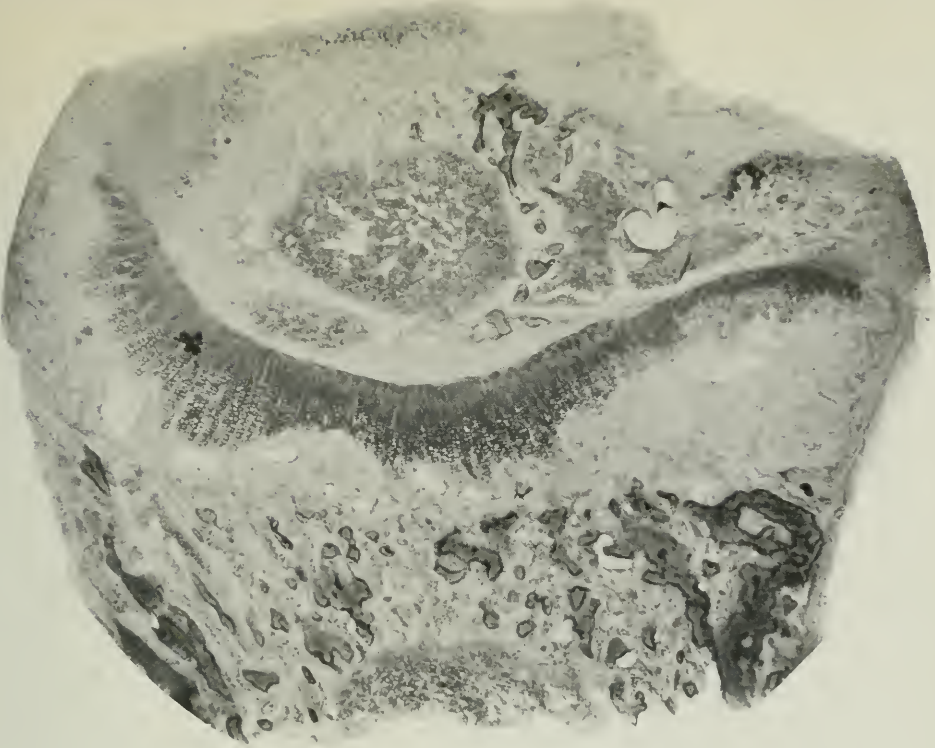

Fig. 3.

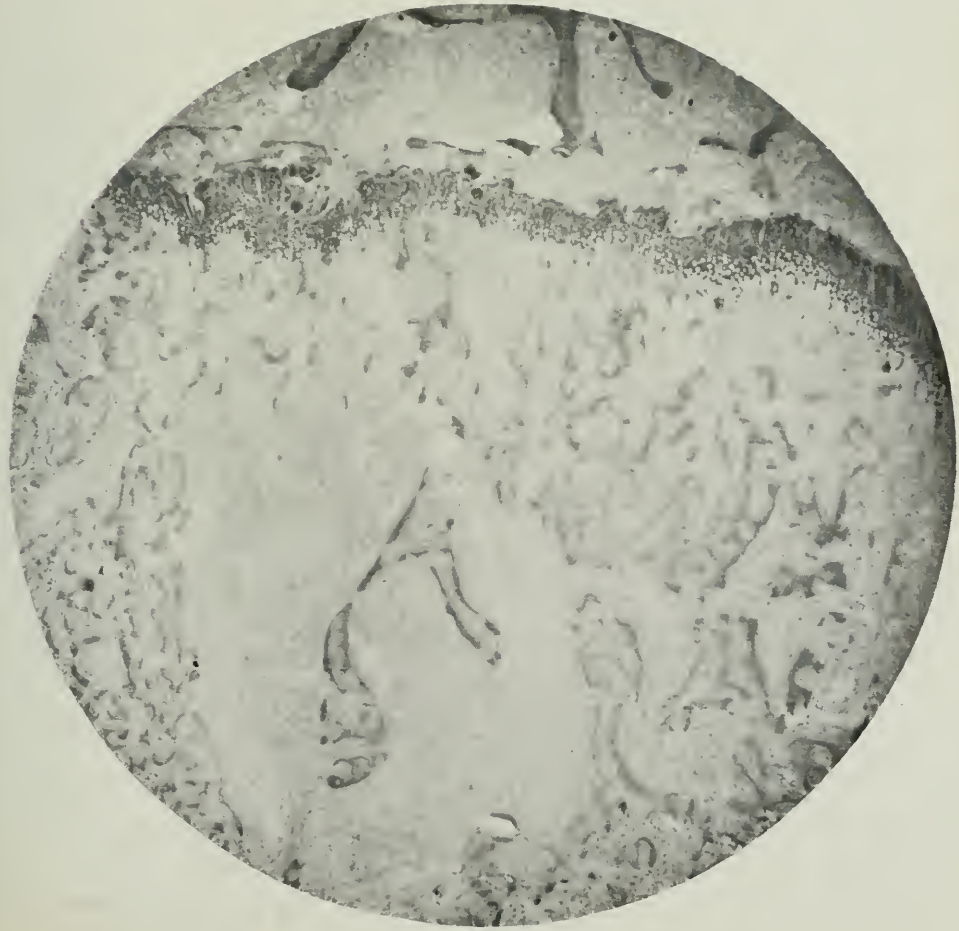

Fig. 4.

PLATE XXXII.

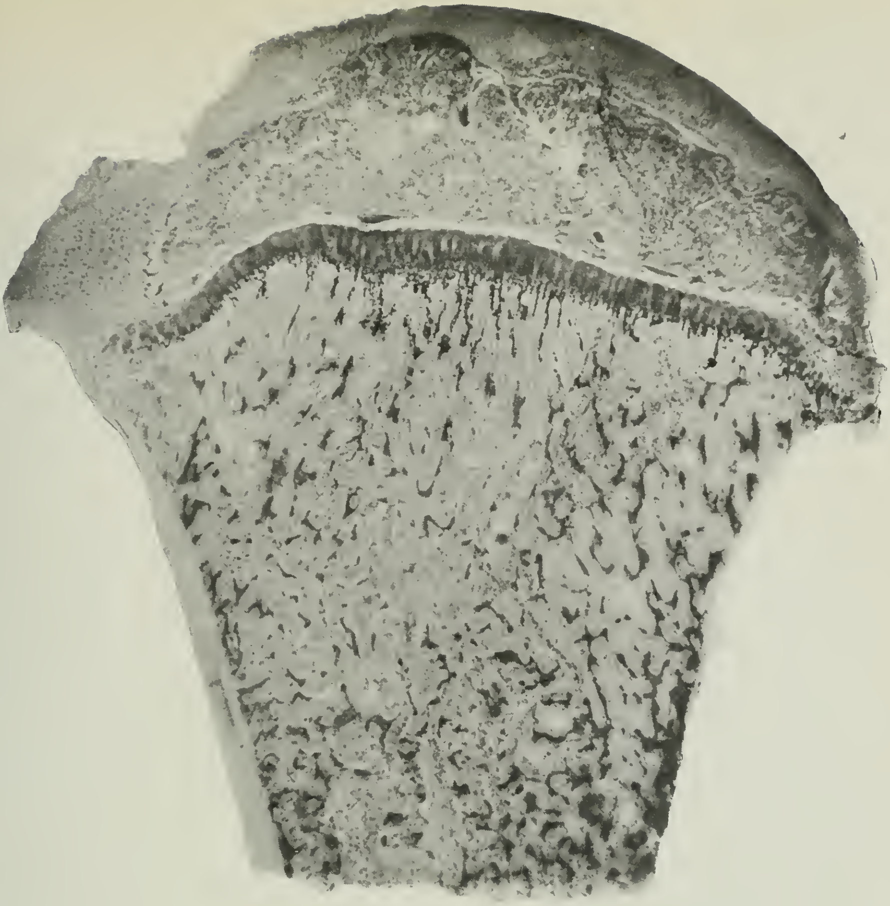

Fig. 5.

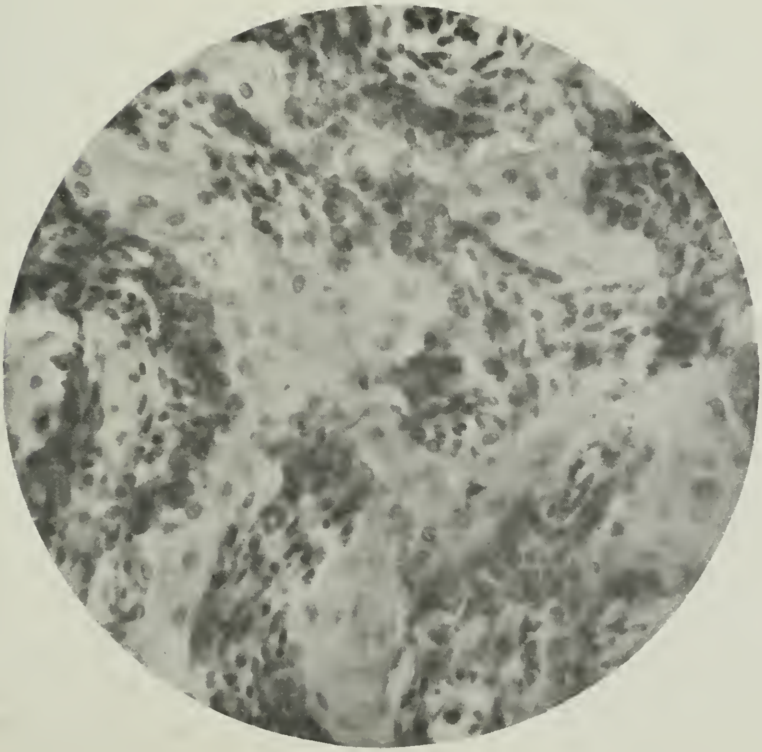

Fig. 6.

PLATE XXXIII.

growth, high fertility and the successful weaning of most of their young. The histological peculiarities of the bones of these rats are illustrated by the microphotograph in Fig. 3.

PLATE XXXI.

FIG. 2. This photograph illustrates the superior physical development and appearance of a group of rats fed the same diet as that of Lot 2821 (Fig. 1 and Chart 5), except that two per cent. of the butter fat in the latter was replaced by cod liver oil. The nature of the faults in the diet are described in the legend to Fig. 1 and that of Chart 5.

The bones of these animals were not far from normal in histological structure. The second generation had bones more different from the normal than were those of the first generation.

The appearance of these animals was strikingly superior to those restricted to the same diet containing 5 per cent. of butter fat. Cod liver oil contains some organic substance which has a pronounced anti-rhachitic effect, and protects young animals against the harmful effects of calcium deficiency and of unfavorable ratios between calcium and phosphorus.

PLATE XXXII.

FIG. 3. The distal end of the femur of a rat on a diet (No. 2810) low in calcium, but containing an amount of butter fat more than sufficient (5 per cent.) to protect the animal against keratomalacia. The cartilage is not calcified and the shaft of the bone contains large amounts of osteoid tissue.

FIG. 4. The bone shown in this figure is from an animal on the same diet (No. 2732) as that which furnished the bone in Figure 4, except that this animal received 1 per cent. cod liver oil instead of 5 per cent. of butter fat. The bone is somewhat osteoporotic, but both cartilage and trabeculae are heavily calcified and there is no osteoid present.

PLATE XXXIII.

FIG. 5. Lower end of the femur from a second generation animal on the diet of Lot 2733, to show the large number of very cellular trabeculae which are only partially calcified.

FIG. 6. Higher magnification of a portion of the bone shown in Figure 5. Note the extreme slenderness of the trabeculae and the large size and number of the osteoblasts, as well as the character of the osteoid tissue.

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ON THE LONGEVITY OF HUMAN INTESTINAL PROTOZOAN CYSTS.*

By WILLIAM C. BOECK, Ph.D.

(Received for publication, July 1, 1921.)

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INTRODUCTION.

The period of viability or longevity of the intestinal protozoan cysts of man is directly related to the period of infectivity of the cysts, and the prophylaxis of protozoan infections. After something is known of the length of life of these encysted organisms other experiments should be undertaken to determine their period of infectivity, i.e., the period during which, should the cysts be ingested, they are capable of producing an infection. But in the absence of knowledge of the period of infectivity, we may assume that while there is life, there is probable ability to produce infection, and hence the period of viability becomes of primary importance. It also follows that when the longevity of the cysts is known in full or in part, it will be possible to adopt effective prophylactic measures to combat the spread of protozoan infections.

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Before introducing the evidence regarding the longevity of the human intestinal cysts, it seems advisable to describe the changes through which the protozoan cysts pass when they are kept for a long time in a favorable environment, i.e., one which is conducive to long life, and when they are present but a short time in an unfavorable environment, in order that such changes may not be mistaken for signs of death in all cases, for some of these changes are only degenerative phenomena.

In connection with some experiments to determine the thermal-death point of the human intestinal protozoan cysts of *Endamoeba histolytica, Endamoeba coli, Giardia intestinalis* and *Chilomastix mesnili* (Boeck, 1921), it was decided to determine the fate of the cysts which had been killed by exposure to high temperature, and which stained with eosin (*dead*), and also the fate of the unstained cysts (*alive*) which were kept in bottles containing distilled water in a cool place, at a temperature of 12° to 22° C. As a result of these observations which were made over a period of 9 months, it was found that the stained cysts of *Endamoeba histolytica, Giardia intestinalis* and *Chilomastix mesnili* underwent extensive plasmolysis, the rate of which was very fast in *Giardia* cysts and slow in the cysts of *Chilomastix* and *Endamoeba histolytica.* As disintegration progressed only a small mass of protoplasm occupied the cysts, and this ultimately disappeared leaving the empty cyst. The cyst membrane too, disintegrated in time, leaving no trace of the cyst.

In the stained cysts of *Endamoeba coli,* a slight plasmolysis first took place, and this was followed by an alteration of the cytoplasm, to form 3 distinct zones (Fig. 1), viz., a central zone or core of protoplasm which usually contained all the nuclei, a narrow peripheral zone which appeared membranous and very coarsely granulated, and a middle zone which was almost without structure, as if fluid and separating the peripheral and central zones. Later on the peripheral zone disappeared as such and took on the appearance of the middle zone. The central core remained for a time, but later underwent dissolution too, leaving the cyst empty.

All these changes were held to be concomitant with degeneration, and their progression accomplished the complete disintegration of the cysts and their disappearance. These observations proved that all cysts which stain with eosin-solution are dead.

But the disintegrative changes just described were not peculiar to stained cysts alone, for as time went on it was found that many cysts which did not take up the eosin stain, underwent similar degenerative changes as those which occurred in stained cysts. This was true for all the different species of cysts.

It was first noticed that the nuclei became distinct, that the cytoplasm appeared more coarsely granular and that slight plasmolysis took place. Later on plasmolysis increased, and in the *Endamoeba coli* cysts the 3 characteristic zones developed as described above (Fig. 1). The final stages of degeneration were similar to those which occurred in the stained cysts. These degenerative changes took place in cysts which had been kept in a favorable environment (in a bottle containing distilled water) for several months, and at all times they refused to take up the eosin stain.

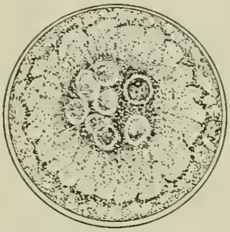

FIG. 1. *E. coli* cyst (dead). × 1860. Degeneration has resulted in the presence of the 3 zones in the cytoplasm.

But again, similar changes occurred within only a few days, when the cysts were in an unfavorable environment such as a stool not washed, or in cultures, in both of which there was an abundant bacterial growth resulting in the elaboration of putrefactive products of decomposition which hastened the degeneration of the cysts and led to the latter's early death.

From the work of Yoshida (1920) on the reproduction *in vitro* of *Endamoeba tetragena (histolytica)* and *Endamoeba coli* from their cysts, we note that many of the cysts which excysted had degenerated to a great extent and had plasmolized, for the cysts were described as possessing "a space, small or large . . . between the outer membrane and the cytoplasm," the dimensions of which varied greatly, "the largest observed being about half the size of the cysts." Certainly such changes were signs of degeneration similar to those observed in the cysts from the bottles, both those which stained with eosin, and those which remained refractive.

The question then arose, are all cysts alive which show marked degenerative changes and do not take up the eosin stain? This question is answered by making further reference to the experiments of

Yoshida. Just as 3 zones occurring in degenerated cysts of *Endamoeba coli* were noticed by the author, so were cysts possessing these changes found by Yoshida in his cultures (see Yoshida, 1920, pl. 29; Figs. 1, 11, 12). On one occasion, he observed the escape of the central core of protoplasm in one of these degenerated cysts upon the removal of the cyst wall. He questioned the viability of the freed protoplasmic mass, presumably one is led to believe, from the fact that it was motionless, as contrasted with the other motile excysted amoebae in his cultures.

We are led to conclude, therefore, that those cysts which show such marked degenerative changes, as very extensive plasmolysis and the possession of only a central core of cytoplasm, and in *E. coli* cysts 3 or less protoplasmic zones of degeneration, are dead (altho they do not stain with eosin, as Yoshida observed), for were such cysts alive then the one which Yoshida saw excyst, would have liberated a motile amoeba as many of his other cysts did which no doubt had not degenerated to the same extent.

This bit of experimental evidence furnishes the best answer to date, to the question whether or not all dead cysts stain with eosin-solution. And when we consider that Yoshida found that most of the amoeba obtained in his cultures were the result of excystation which was characterized by a "disintegration and dissolution of the cyst wall," we are also furnished with data to prove that cysts may undergo degeneration to the extent that their nuclei become visible, their cytoplasm more coarsely granular and less refractive, and that they may undergo some plasmolysis and still be viable and capable of excystation, but that those cysts are dead in which more extensive degenerative changes occur, such as alteration of the cytoplasm into zones as in *Endamoeba coli,* and plasmolysis to the extent that only a central core of protoplasm remains in other species. We can look upon slight changes as early degenerative phenomena, but upon marked changes as certain signs of death, even altho these changes occur in cysts which do not stain in the eosin-solution.

CRITERION OF VIABILITY.

All the data regarding the longevity of the cysts which will be presented in the following portion of this paper were collected with the understanding that those cysts were *alive* which did not stain in eosin-solution and showed but little or no plasmolysis and little or no loss in refractivity, while those cysts which stained in eosin-solution or remained unstained, but were markedly degenerated as described above were held to be *dead*. By supplementing the eosin-criterion of viability in this way with direct observation on the morphological changes which were present, it is believed that an almost certain differentiation was possible between dead and living cysts.

TECHNIQUE.

It has been shown by Kuenen and Swellengrebel (1913) and Boeck (1921) that the presence of bacteria and the chemical products of putrefaction exert a deleterious influence upon the vitality of the cysts; the different species of protozoan cysts were accordingly prepared so as to be comparatively free from these factors, by washing the stools containing the cysts.

The washing of the stool was accomplished as follows: The stool was immersed in distilled water and comminuted with a mechanical mixer. It was then strained through a couple of layers of bolting-cloth which removed the coarse fecal matter. The filtrate was allowed to stand for a few hours, during which time the cysts fell to the bottom of the container. The supernatant fluid containing bacteria and water-soluble putrefactive products was drawn off and more distilled water added. This procedure was repeated several times until the supernatant fluid was no longer colored or turbid, and then the cysts were transferred to a bottle with 50 to 100 c.c. of distilled water, and kept in a cool place where the temperature varied from 12° to 22° C. This low temperature inhibited excessive bacterial growth and retarded putrefaction to the point that it might be considered negligible.

The longevity studies were made upon the cysts which were kept under 2 different environments, (1) during the time they were kept in distilled water in their respective bottles and (2) while present in vaseline-slide preparations. In both environments the temperature was the same, 12° to 22° C. They were not exposed to sunlight nor kept in total darkness.

When examinations were made of the cysts from the bottle, sediment containing the cysts was withdrawn and a smear made in eosin-solution (1 : 1000 aqueous) to aid in differentiating the living from the dead cysts. Vaseline-slide preparations were made by taking a little sediment containing cysts from each of the bottles, making a smear in eosin-solution and sealing the specimens with vaseline to prevent evaporation. In the former case different cysts were observed at each examination since new preparations were made from the bottles for every observation, but in the latter case, the same groups of cysts were observed from time to time, as new vaseline-slide preparations were not made for each observation, but the original ones kept.

Cysts in bottles.

The results of the observations made upon the cysts in the bottles are given in Table I. Only the date of the last examination is given (4th column) on which viable cysts were encountered; how much longer these cysts remained viable was not determined. The number recorded in the 5th column represents the number of cysts which were viable out of the first 50 cysts seen in the examination. This number may be multiplied by 2 to obtain the per cent. of the cysts which were still living. In the 6th column is given the number of days which had elapsed since the cysts were passed with the feces i.e., their age.

TABLE I.

Longevity of the cysts in distilled water, in bottles.

Cysts.	Date passed.	Date stool washed.	Last examination.	No. cysts viable.	Age in days.
E. histolytica a	Dec. 8, 1919	Dec. 11, 1919	Apr. 26, 1920	16	139
b	" 14, 1920	" 15, 1920	May 16, 1921	1	153
E. coli	Jan. 15, 1920	Feb. 5, 1920	Sept. 16, 1920	6	244
Giardia intestinalis a	Oct. 18, 1919	Oct. 22, 1919	Nov. 14, 1919	1	27
b	Oct. 18, 1919	Oct. 22, 1919	Nov. 19, 1919	32	32
c	Aug. 6, 1920	Aug. 6, 1920	Aug. 26, 1920	4	20
Chilomastix mesnili	Jan. 27, 1920	Jan. 27, 1920	Aug. 2, 1920	44	187

From a comparison of the number of viable cysts, and the number of days elapsed since their passage, it is apparent that the cysts of *Chilomastix mesnili* might be expected to live the longest, for 88 per cent. of these were viable when they were 187 days old. This was no doubt due to the greater resistance of the *Chilomastix* cysts compared to the others, to the lower temperatures of 12° to 22° C., as was pointed out in the experiments to determine the thermal-death point of intestinal protozoan cysts (Boeck, 1921). In all probability the proportion of 44 viable cysts out of the first 50 cysts counted is higher than it should be because the empty cysts of *Chilomastix* are very difficult to see in a smear preparation on account of their small size and thin cyst wall, and they are therefore easily overlooked in an examination. In other words the death rate of these cysts was perhaps greater than the figures in Table I indicate. The cysts of *E. coli* were next in length of life, for 6 were alive 244 days after their passage from the body. The *E. histolytica* cysts were very resistant too, for 16 cysts had remained viable for 139 days. In the *b* series performed a year later, one viable cyst was seen 153 days after its passage in the stool on Dec. 14th, 1920. This cyst did not stain in eosin-solution; it had not undergone plasmolysis, and but a slight loss in refractivity. The nuclei were still invisible which is very unusual.

The *Giardia* cysts were not so long-lived. Three different series of observations were made upon cysts kept in bottles. One bottle of cysts from which the *b* series was made, was kept out of doors where the temperature was somewhat lower. To judge from the *a* and *c* series the *Giardia* cysts could not be expected to live much longer than 27 days, but in the *b* series (which was kept out of doors), they might have lived several days longer. This no doubt can be attributed to the influence of the cooler temperature out of doors which kept down the growth of the bacteria more effectively than the temperature indoors.

Cysts in vaseline-slide preparations.

Vaseline-slide preparations were kept at the same temperature of 12° to 22° C., at which the cysts in the bottles were kept. These preparations made it possible, as has been said above, to observe the same group of cysts from one examination to another, and furthermore to exclude all outside factors of influence from entrance. In these preparations the bacteria were greatly inhibited in their multiplication for the medium never became turbid, in fact it remained remarkably clear at all times.

Again, as in the above observations, the number of viable cysts out of the first 50 cysts encountered is recorded in the 5th column of Table II. The 6th column gives the age of these cysts dating from the passage of the stool. How much longer these cysts remained viable was not determined. They all showed a loss of refractivity and visible nuclei, but nevertheless they did not take up the eosin stain and the disintegrative changes were not such as to indicate that they were dead on the day of the last examination (Table II, column 4).

TABLE II.

Longevity of the cysts in the vaseline-slide preparations.

Cysts.	Date passed.	Date of vaseline-slide.	Date examined.	No. of cysts viable.	Age in days.
E. histolytica	Dec. 8, 1919	Feb. 5, 1920	July 21, 1920	12	211
E. coli	Jan. 15, 1920	Feb. 12, 1920	May 17, 1920	9	124
Giardia intestinalis a.	Oct. 18, 1919	Oct. 24, 1919	Dec. 23, 1919	10	66
b.	Aug. 6, 1920	Aug. 6, 1920	Sept. 7, 1920	2	22
Chilomastix mesnili	Jan. 27, 1920	Feb. 6, 1920	Sept. 16, 1920	45	232

From a study of Table II, it will be seen that the *Chilomastix* cysts again proved themselves able to live longer than the other species of cysts. And again, it may be said, that the proportion of viable cysts out of the first 50 cysts observed is no doubt higher than it should be for the same reason as given above, namely that empty cysts are easily overlooked. Nevertheless it was apparent that these cysts were out-living the others, and this (possibly?) by reason of their strong resistance to the lower temperatures.

It was a surprise, that the cysts of *E. histolytica* lived longer in the vaseline-slide preparations than those of *E. coli*. I believe, however, this does not actually occur in life, but that the opposite is true instead, as was found to be the case with the cysts in the bottles. It must have been that the *E. coli* cysts in the vaseline-slide preparations were affected by some detrimental influences either before the preparations were made, or afterward, that resulted in their early death.

Again, the *Giardia* cysts were shown to die at an early age compared to the others, but it is significant that 10 cysts were still viable at the end of 66 days (series *a*), and this was considerably longer than the cysts remained viable in the bottles. In another series, *b* (Table II), the cysts lived but little longer than 32 days, in all probability, and this, in view of the data in the *a* series can only be attributed to the influence of some detrimental factor. It is known for instance, that urine has a decidedly deleterious effect upon these cysts especially, and it is conceivable that it was previous contact with this factor while in the stool' or some other influence, which resulted in the early death of these cysts in the *b* series.

Survival of cysts in fly's intestine.

Root (1921) has shown that cysts of the human intestinal protozoa may remain apparently viable for many hours in the intestine of the fly. It seems significant to point out that those cysts which survived the longest in the fly's intestine were of the same species as those which were found to live the longest in the experiments and observations given above. The period of survival of the cysts in the fly's intestine was proportional to their longevity outside when immersed in distilled water, altho the length of life of the cysts in the intestine was ever so much shorter. For the sake of convenience in comparing the results which Root obtained with those on longevity the following table is given. The number of viable cysts out of the first 50 observed is recorded at the time of the last examination.

TABLE III.

Period of viability of cysts in fly's intestine, as compared with their survival in distilled water in bottles and in vaseline-slide preparations.

	In fly's intestine.		In bottles or vaseline preparations.	
	No. cysts viable.	End of	No. cysts viable.	End of
Giardia intestinalis	1—(1 series)	16 hours	10	66 days
E. histolytica	1—(1 series)	49 “	12	211 “
E. coli	1—(1 series)	52 “	6	244 “
Chilomastix mesnili	2—(1 series)	80 “	45	232 “

It is to be seen at once that the cysts of *Giardia intestinalis* live the shortest of all the cysts, whether in the fly's intestine or outside when immersed in distilled water. Then follow the cysts of *E. histolytica, E. coli* and lastly *Chilomastix mesnili.*

Assuming that the factors in the intestine of the flies used in the experiments by Root equally influenced all the species of cysts after their ingestion, the period of survival becomes an expression of the comparative resistance of the different species of cysts. Of all the factors present in the intestine of the flies, fermentation caused by the bacteria in the intestine was probably chiefly responsible for the death of the cysts, as the temperature was low, in as much as this tends to be the same as that of the surrounding atmosphere, therefore room temperature (27° C.). It is altogether likely that the digestive juices may also have operated against the survival of the cysts. Therefore we may rightly assume that the cysts of *Chilomastix mesnili* were more resistant than the other species of cysts, in view of the sameness of the environment. The *Chilomastix* cysts survived longer than the other cysts in the fly's intestine, very probably on account of their greater resistance to the low temperature (27° C. or less), all other factors being equal, as was shown to be the case in the thermal-death point determinations (Boeck, 1921). For this same reason, in all probability, the *Chilomastix* cysts lived longer than the other species in the experiments given above to determine their longevity.

Intracystic development.

There was no evidence of definite intracystic development in the viable cysts which had been kept in the supply bottles and in the vaseline-slide preparations. In the observations made upon the endamoebic cysts, no evidence whatsoever occurred to indicate that single nucleated cysts developed into bi-nucleated cysts and binucleated cysts into 4-nucleated cysts and so on, as was thought to occur by some of the earlier investigators.

I believe that the finding of 1 and 2-nucleated cysts of *E. histolytica* in the feces as well as of 1, 2, and 4-nucleated cysts of *E. coli* only signifies that these cysts were prevented from reaching the full development of 4-nucleated cysts in the case of the former parasite and of 8-nucleated cysts in the latter, by their premature ejection from the human body and that they develop no further unless their environment closely simulates that of the human intestine, a possible exception being those cysts in which the nuclei are in the midst of mitosis, when they have become transformed into spindles.

In view of the longevity observations described above it seems that the reproduction *in vitro,* as described by Yoshida (1920) for *E. coli* and *E. histolytica,* represents abnormal or degenerative phenomena brought about by the fermentative action of the bacteria in his artificial cultures, causing the newly formed amoebae to die on the first or second day following excystation "from the effects of putrefaction in the culture medium." It is only reasonable to assume that the bacteria were responsible for the disintegration of the cysts by progressive plasmolysis, some of which excysted to give rise to abnormal amoebae.

Although I was unable to detect any development in the cysts of

Giardia and *Chilomastix,* failure in the latter case must have been due to some unknown cause, since Kofoid and Swezy (1920) found that many *Chilomastix* cysts underwent development after they had been passed from the human body. The development resulted in the mitotic division of the nucleus and in the formation of another neuromotor apparatus. The only changes I saw, however, in the *Chilomastix* cysts which had been kept for 232 days was the presence of small, usually spheroidal-shaped chromatin-staining masses in the cytoplasm. These masses occupied no definite position in the cysts, but were situated here and there. Generally they were from 1 to 4 in number. The origin of these bodies was not indicated. They may represent chromatoidal bodies, the remains of the disintegrated neuromotor apparatus, or what is less probable chromatin material which had been extruded into the cytoplasm from the nucleus. The nucleus in these cysts possessed a large central karyosome, or at times, 1 to 3 smaller chromatin masses. A centrosome was demonstrable at the anterior pole of the nucleus on the nuclear membrane. The neuromotor apparatus had partly disappeared in many cysts, for not all the structures were visible which go to make up this fibrillar complex.

Period of infectivity.

With the knowledge at hand that the cysts of the human intestinal protozoa may remain viable for several months as has been shown with the use of the best criteria of viability available, it is of utmost importance to determine the period of infectivity as was mentioned in the opening paragraph of this paper. In all probability the period of infectivity does not coincide with the longevity of the cysts, but the proof is very meager at present to substantiate this statement. The most valuable evidence we have pertains to ingestion experiments upon man and kittens with cysts of *Endamoeba histolytica, Endamoeba coli* and *Giardia intestinalis.*

Darling (1913) after diluting the feces containing cysts of *E. histolytica* with 10 volumes of sterile tap water and allowing them to stand for 3 days, was unable to infect 2 kittens. But this might have been due as he states, "to an insufficiency of cysts."

Walker and Sellards in 1913, however, were able to infect men with *E. histolytica* with cysts which had been kept for 2 days at tropical temperature, and with *E. coli* with cysts which had been kept 10 days. The cysts were kept moist in both cases.

Other investigators have made observations on the longevity of the intestinal protozoan cysts of man, among them Penfold, Woodcock and Drew (1916), who kept the cysts of *E. histolytica* alive in running water for 15 days; Thomson and Thomson (1916), who observed viable cysts of *E. histolytica* in moist feces after 16 days, and Wenyon and O'Connor (1917) after a month, but none of these workers conducted much needed experiments to see whether the viable cysts were also infectious for man or laboratory animals.

The experiments of Walker (Sellards) in 1913 were conducted upon human beings and his results therefore are especially noteworthy. It seems probable, however, in view of the fact that cysts may be kept apparently viable for a long time that the period of infectivity for *E. histolytica* would be found longer than 2 days and for *E. coli* longer than 10 days had more experiments been conducted of a similar nature to those performed by this investigator.

The period of infectivity of *Giardia intestinalis* is considerably longer than that known thus far for the entamoebic cysts; it appears to coincide with the period of viability. Fantham and Porter (1916) were able to infect kittens with cysts of this parasite which had been kept for 74 days after they had been washed free from fecal debris. This period of infectivity is even greater than the longest period of viability, which was 66 days, obtained in the experiments detailed above, in the *a* series of Table II.

Until we are in possession of more definite facts regarding the period of infectivity of all intestinal protozoan cysts of man, and especially of *E. histolytica*, all prophylactic measures should look toward the destruction of the feces within 2 days if possible, since as we have seen, cysts may be still infectious within this period, if ingested.

Man contracts his infection through accidental contact with the feces containing the cysts and ingesting the latter or probably through the eating of food or drinking of water in which cysts have been deposited by flies which have visited privies and eaten feces containing cysts. These methods of dissemination emphasize the importance, as other investigators have shown, of adopting such preventative measures of sanitation as will exclude the flies from the feces, and also to dispose of the latter as soon as possible after defecation.

SUMMARY.

1. Accepting the eosin-solution, supplemented by morphological observations as a criterion of viability, the longevity was determined for 4 different species of human intestinal protozoan cysts, under 2 different environments.

2. Immersed in distilled water, contained in bottles and kept at a temperature of 12° to 22° C., cysts of *E. histolytica* were found viable at the end of 153 days; *E. coli* at the end of 244 days; *Giardia intestinalis* at the end of 32 days and *Chilomastix mesnili* at the end of 187 days.

3. In eosin-stained wet preparations, sealed with vaseline, cysts of *E. histolytica* were viable at the end of 211 days; *E. coli*, 124 days; *Giardia intestinalis,* 66 days and *Chilomastix mesnili* at the end of 232 days.

4. While the results of the observations did not reveal the actual length of life of each species of cyst, yet they demonstrated that the cysts remained viable for a considerable period of time and that under similar conditions of a low temperature and the exclusion of bacterial influences, the cysts of *Chilomastix* will probably live the longest, due (possibly?) to their stronger resistance to the lower temperatures.

5. The period of infectivity of the human intestinal protozoan cysts is not definitely known and more experiments should be made to determine it. It has been shown, however, that the cysts of *E. histolytica* and *E. coli* are infectious for man 2 and 10 days respectively, after their passage from the human body, and the cysts of *Giardia intestinalis* for kittens, after 74 days.

6. In the absence of more definite information regarding the period of infectivity, all prophylactic measures against the dissemination of protozoan diseases should aim to prevent the access of flies to feces and to destroy the feces within 2 days after their defecation.

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DISINFECTING SKINS AND HAIR FOR ANTHRAX.

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Introduction.

In the fall of 1917 the author at the request of a glazed kid tanner in Philadelphia made some studies of the action of salt and hydrochloric acid on anthrax spores, in conjunction with tests made at the tannery to determine the possibility of making satisfactory leather from hides treated by this method, the so called Schattenfroh method of disinfecting hides. These tests were repeated and carried somewhat further the following year by a group of students. In the fall of 1920 at the request of the Pennsylvania State Department of Labor a survey was made of the Horse Hair Dressing Industry in Philadelphia during which virulent anthrax organisms were isolated from imported dressed China horse hair. At the same time the Schattenfroh method was again tested, and other studies made of the resistance of anthrax spores to heat and to the action of cyllin, an English proprietary disinfectant with a rated phenol coefficient of 11 (R-W). Tests with cyllin were made because it is used in one of the methods certified in England, and from what we can learn it seems to be very widely used there for the disinfection of horse hair. Up until September 1920 at least, the cyllin certificate had not been withdrawn although Dr. T. M. Legge informs me that it is realized that its action is uncertain.

In view of the increasing incidence of industrial anthrax in this country and the paucity in American scientific literature of reports on detailed tests of methods of *destroying* anthrax spores it seemed advisable to publish the results of the above series of tests. All of the tests will not be given in detail, but the methods employed and the results obtained will be outlined, and the more important and conclusive tests will be given in full.

In selecting a method for the disinfection of hides two very important factors must not be overlooked. The method must be sufficiently economical to be useful commercially, and the disinfected hide must not be so altered as to render it unfit for tanning for leather. One or the other of these conditions prevent the employment of a number of otherwise practical and fairly sure methods, and limits very considerably the choice of available disinfection processes, such as the use of steam under pressure or of formalin solution, both of which produce untannable hides.

Among the methods of disinfection recommended for hides infested with anthrax there are two that have received special mention and have been extensively tested abroad and less so in this country. The Seymour-Jones method (1) consists in soaking hides for 24 hours in a 1 to 5000 bichloride of mercury solution containing 1 per cent. of formic acid, followed by a short soak in a saturated salt solution. This method was not tested, as from the reports of others the sterilization at the end of the period of exposure is more apparent than real. Among others Tilley (2) in this country and Hilgerman and Marmann (3) abroad showed that if the treatment is followed immediately by a neutralization with ammonium sulphide or sodium sulphide it is usually possible to recover anthrax organisms from treated skins. Tilley found that spores were not all killed in 24 hours by a 1 to 250 bichloride solution with 1 per cent. formic acid, but that even the weaker solution recommended by Seymour-Jones was fairly effective if neutralization was postponed several days. The Bureau of Animal Industry regulations (4) permit the use of this method provided the hides are held for two weeks after treatment before neutralization or further use.

From the published results of tests the Schattenfroh method (5) seemed to give greatest promise, and this was the method first tested by the author. The earliest tests were made with solutions prepared by the tanner for whom the testing was undertaken, so the percentages of acid present cannot be vouched for, but later tests were made from carefully prepared dilutions of Baker analysed acid, and were based on the recorded content of anhydrous hydrochloric acid. Schattenfroh's original paper was published in 1911, and his method has been thoroughly tested out and reported upon by several European workers (3, 5, 6, 7). The most extensive report on the use of this method in this country is that by Tilley (2), and the author has been unable to find any other American report giving any details of methods used in tests. Osborn (8) states that experiments by Dr.

F. H. Slack of the Sias Laboratories, carried out for a leather firm in Massachusetts, under practical conditions showed that 10 per cent. NaCl and .75 per cent. HCl is on the border line of successful disinfection, and that 1 per cent. HCl and 10 per cent. NaCl is satisfactory for killing anthrax spores on hides, if the temperature is maintained at 60–70° F. for 40 hours, but gives no details of test methods. He recommends 2 per cent. HCl and 10 per cent. NaCl for commercial use. Hubbard (9) says, referring to both the Seymour-Jones and Schattenfroh methods, that "our laboratory informs us that these methods will destroy spores; but until they are proved by extensive use, they cannot be pronounced absolutely safe," and he also gives no details of tests on which these statements are based.

CULTURES USED IN TESTS.

In the tests here to be reported it was attempted to approach as far as possible practical working conditions in the tannery and the hair industry. Realizing that there is more or less variation in resistance of spores from different strains of the same organism several cultures of anthrax were used. Culture No. 1 was an old laboratory strain, grown on artificial media for over five years before the first tests were made. Cultures 3, 4 and 5 were isolated in 1917 from three out of five samples of tannery soak (from five soak vats in one tannery) by means of guinea pig inoculation; and culture 43 was isolated in 1920 from a sample of dressed horse hair from China and used in the later tests reported.

Table I shows the varying resistance of four of these cultures to heat and to several germicides. These tests showed no appreciable difference in the resistance of the strains tested to anything except calcium oxide, in the strength permitted by the Bureau of Animal Industry regulations (4) for sheep and goat skins. The Bureau permits the use of a 12 hour soak in milk of lime (5 per cent. CaO), and these tests showed that only one of four strains was killed in 48 hours in this strength, while two of the four survived 72 hours. This is not surprising as Hilgerman and Marmann (3) report that they found in tests of liming of tannery wastes, to destroy anthrax, that such waste to insure destruction of all spores should contain at least 20 per cent. of CaO and should not be used as fertilizer for at least 3 months.

TABLE I.

Resistance tests of anthrax spores.

Strain No.	Source.	Grown on.	Sub-culture in.	Boiling in Bouillon.	Autoclave at 10 lbs.	5% Phenol.	1% Tricresol.	1–1000 Hg.Cl$_2$.	5% CaO.
1.........	Laboratory stock	Nutrient agar + 1.5%	Bouillon + 1.5%	Killed in 25 min.	Killed in 10 min.	Killed in 20 min.	Survived 72 hrs.	Survived 20 min.	Survived 72 hrs.
3.........	Tannery vat.	Nutrient agar + 1.5%	Bouillon + 1.5%	Killed in 25 min.	Killed in 10 min.	Killed in 21 min.	Survived 72 hrs.	Survived 20 min.	Survived 72 hrs.
4.........	Tannery vat.	Nutrient agar + 1.5%	Bouillon + 1.5%	Killed in 25 min.	Killed in 10 min.	Killed in 20 min.	Survived 72 hrs.	Survived 20 min.	Killed in 72 hrs.
5.........	Tannery vat.	Nutrient agar + 1.5%	Bouillon + 1.5%	Killed in 25 min.	Killed in 10 min.	Killed in 21 min.	Survived 72 hrs.	Survived 20 min.	Killed in 48 hrs.

TESTS WITH THE SCHATTENFROH METHOD.

The first tests were made by cutting up dried goat skin, with the hair on, into pieces about 2 centimeters square, sterilizing in the autoclave and then covering in tests tubes with a bouillon suspension of the different strains of anthrax organisms and incubating for 3 days. Stains made at the end of this period showed abundant spore formation. The bouillon was then drained off and replaced by different strengths of acid and salt solution, there being about a dozen squares in a tube and just enough solution to cover them. The tubes were then kept at room temperature, and squares fished out at 24 hour

TABLE II.

Tests of Schattenfroh method.

Strain No.	Source.	Culture on	% HCl.	% NaCl.	Sub-culture in bouillon after				
					1 day	2 days	3 days	4 days	5 days
1	Laboratory stock	Agar	3	12	+	–	–	–	–
		"	3	12	+	–	–	–	
		"	3	10	+	+	–	–	
		"	3	10	+	+	+	–	
		"	3	5	+	+	+	+	–
		"	2	5	+	+	+	+	+
3	Tannery vat	"	3	12	–	–	–		
		"	3	12	–	–	–		
		"	3	10	–	–	–		
		"	3	10	–	–	–		
		"	3	5	–	–	–		
		"	3	5	+	+	+	–	–
		"	2	5	+	+	+	+	+
4	"	"	3	12	–	–	–	–	
		"	3	12	+	–	–	–	
		"	3	10	–	–	–	–	
		"	3	10	+	–	–	–	
		"	3	5	+	–	–	–	
		"	3	5	+	+	–	–	
		"	2	5	+	–	–	–	
		"	1	5	+	+	–	–	
5	"	"	3	12	+	–	–	–	
		"	3	12	+	–	–	–	
		"	3	10	+	–	–	–	
		"	3	10	+	+	–	–	
		"	3	5	+	–	–	–	
		"	3	5	+	+	–	–	
		"	2	5	+	–	–	–	
		"	1	5	+	+	+	+	+

+ = Growth.
– = No growth.

intervals for periods of five days, washed in water, dropped in bouillon tubes and incubated for 4 days. These tests showed consistent results, though distinctly less rapid action than reported by other workers or than in tests carried out later, possibly because of the heavy inoculation and possibly because of recontamination of skin samples from spores on the surface of the tube above the acid solution. These tests (see table II) showed two strains killed by 2 per cent. HCl and 5 per cent. NaCl in 2 days, agreeing with Slack's tests (8), but survival of two strains in the same strength soak for 5 days. 3 per cent. HCl and 10 per cent. NaCl apparently killed one strain in 24 hours in two tests, another strain in 24 hours once and 48 hours once, a third strain in 2 and 3 days respectively and a fourth strain in 3 and 4 days. There is a possibility that the acid content of the solutions supplied by the tanner may have been based on the percentage of c.p. liquid hydrochloric acid and not on anhydrous hydrochloric acid, and the results of subsequent tests with solutions prepared by the author would seem to indicate this. In these tests neutralization after exposure was purposely omitted, as the tanner was not neutralizing his hides before transferring to the dehairing lime soak in which the skins remain for some days. It was felt that simple washing would remove acid from the surface and that surface disinfection was all that was necessary, as the long lime soak would kill any live spores remaining in the center of the skin. Hilgerman and Marmann's results quoted above, however, make this assumption very doubtful. On the basis of these tests the tanner proceeded to treat skins by soaking 48 hours at room temperature in 3 per cent. acid and 10 per cent. salt, and to make from treated skins satisfactory glazed kid in spite of the fact that other tanners in the city complained to the Bureau of Animal Industry that it was impossible to make glazed kid from skins so treated. Tests reported by foreign workers claim that good leather can be made from such skins (3, 5, 6, 7), and Osborn (8) says that the process is used routinely in making leather for uppers in Massachusetts. These tests showed an apparently decided difference in resistance to acid and salt with different strains.

Obtaining resistant spores.

In the Archiv für Hygiene, in the summer of 1920, Rupert Reiter (10) reports studies on variations in the resistance of anthrax spores and states that he was able to obtain spores of decidedly increased resistance to streaming steam by preliminary growth on a wheat agar prepared by soaking 500 grms. of wheat grits in a liter of water for 12 to 24 hours, filtering, adding to the filtrate 1.75 per cent. agar, neutralizing and autoclaving. He also quotes from Süpfle and Dengler (11) to the effect that spores damaged by heat and disinfectants could be made to develop in a dextrose-serum-bouillon (3 per cent. dextrose and 5 per cent. serum) when they would fail to develop in ordinary nutrient bouillon. Reiter's tests confirmed this fact. Table III gives the results of tests by the author

TABLE III.

Resistance to streaming steam.

Spore suspensions dried on silk thread. Time in which cultures were killed.

Culture.		Culture on Nutrient Agar.		Culture on Wheat Agar.	
		Sub-culture in		Sub-culture in	
No.	Source.	Bouillon.	Dextrose-Serum Bouillon.	Bouillon.	Dextrose-Serum Bouillon.
1	Laboratory stock	30–35 min.	30–35 min.	35–40 min.	35–40 min.
4	Tannery vat	10–15 "	30 "	35–40 "	35–40 "
5	"	10–15 "	35 "	30–35 "	35–40 "
3	"	10–15 "	15–20 "	20–35 "	35–40 "
43	Chinese horsehair	10–15 "	15–35 "	15–20 "	20–35 "
Extremes..................		10–35 "	15–35 "	15–40 "	20–40 "
Average....................		16.5 "	24.3+ "	30.5 "	35.5 "

serving to confirm both of these statements. In these tests cultures of the 5 strains were grown on wheat agar and on nutrient agar for 3 days, stains demonstrating abundant spore formation in this time in all cases, and the growth was washed off in physiological salt solution and soaked up by sterile white silk threads which were then air-dried at room temperature. The threads were then exposed in open Petri dishes in the Arnold to streaming steam, taking out one thread from each dish at five minute intervals, cutting in half and dropping half in bouillon and half in dextrose-serum bouillon. These tests showed that culture 1 gave most resistant spores on agar, apparently surviving for 30 to 35 minutes when tested in bouillon (2 tests) against a 10 to 15 minute survival of the other four strains. Dextrose-serum-bouillon cultures showed, however, that the other strains had survived for from 15 to 35 minutes. Spores grown on wheat agar proved more resistant and when sub-cultures were made in dextrose-serum bouillon all but the most recently isolated culture survived at least 30 minutes. These tests seemed to show that the author was working with somewhat more resistant spores than did Reiter, as his spores from agar were killed by streaming steam in 5 minutes, while the spores of five strains grown on wheat agar were killed in an average of 26.4 minutes. As a result of these tests all subsequent tests were made with spores obtained from wheat agar, and all subcultures after exposure were made in dextrose-serum-bouillon as well as bouillon. Microscopic examination of cultures showed that wheat agar cultures produced abundant spores earlier than did ordinary nutrient agar. This led to the suggestion that the apparent increased resistance might merely be due to an earlier spore formation permitting the spores to become more resistant. Comparative tests made with spores from 3 and 6 day wheat agar and 6 day nutrient agar failed to show that this was the case.

In tests of anthrax disinfection methods several means have been employed for insuring a satisfactory infestation of test samples. Hilgerman and Marmann used linen squares soaked in saline suspensions from 2 day agar slants, and also cat skins over which the suspension was poured and then was rubbed in with a glass rod. Gegenbauer and Reichel used silk threads and naturally infected hides. Moegle used naturally infected hides. Tilley used squares of hide with a drop of rabbit blood dried on the hide after inoculation with an anthrax spore suspension.

The author preferred to use cultures in which abundant spore formation was demonstrated by staining, and to use moist samples rather than to lower the resistance of the spores by prolonged drying.

Inoculating skin samples with anthrax.

In an attempt to demonstrate the efficacy of the method of inoculating skin samples by soaking in broth cultures, several samples so treated were imbedded in paraffin and sectioned and stained. These sections showed that the anthrax organisms covered the surface and grew into any depressions such as large hair follicles or accidental tears, but that they failed to penetrate into the intact hide. This showed that skin so treated, while it probably was decidedly more heavily infested with organisms on the surface than would be skin from an animal dying of anthrax, had no organisms under the surface as would be found in the skin capillaries in the latter case. This led to a change in the method of inoculation. Pieces of skin about 4 centimeters square were placed hair side down in Petri dishes after having been soaked several days in sterile water to soften. A very turbid suspension of spores from wheat agar cultures was then injected hypodermically under pressure diagonally into the skin. As the needle was withdrawn a pool of spore suspension was deposited on the surface and numerous oblique punctures through the suspension into the skin were made. These pieces of skin were then covered with Schattenfroh's acid-salt solution, and after 40–48-hour exposures at room temperature were washed in water, cut in half with sharp scissors to expose any centrally located spores and dropped in bouillon and dextrose-serum-bouillon.

Details of later Schattenfroh tests.

In all tests except those already tabulated the dried skin samples were weighed and covered with acid-salt solution in a definite proportion of solution to dry skin. Being informed that tanners usually used 1000 gallons of water to 1200 pounds of skin this ratio was approximated by placing 3 grams of skin in 25 c.c. of solution in Erlenmeyer flasks. This was decidedly less skin per cubic centimeter than used in the first test and possibly explains why in all subsequent tests 1 to 2 c.c. of HCl with 5 c.c. NaCl was sufficient to kill anthrax in 48 hours.

Table IV gives the results of tests so made with different strengths of acid and salt. In these tests a mixed suspension of spores from five strains of anthrax was used. They confirmed similar tests made with skin samples soaked in cultures of the separate strains of organisms. The strengths of acid and salt used were those used by Slack, as reported by Osborn.

As Schattenfroh's method called for the use of 2 per cent. HCl and 10 per cent. NaCl, and as this is the strength recommended and used in Massachusetts, final tests with this strength of solution were made, and as directed by Schattenfroh, the treated skin samples, after the 40–48-hour soak were neutralized for one half hour in a 4 per cent. sodium carbonate solution, to prevent any trace of acid being carried over into the cultures. After neutralization they were washed in water until the wash water would not react alkaline to litmus, and then were cut in half and planted in bouillon and dextrose-serum-bouillon. A control with a sample inoculated with cul-

TABLE IV.

Schattenfroh tests.

Squares of skin soaked in sterile water and then injected intradermally with mixed anthrax spores suspension.

	Soak 40 hrs. in %		Sub-culture in	
	HCl.	NaCl.	Bouillon.	Dextrose-serum Bouillon.
Spores grown on nutrient agar.....	2	10	—	—
	2	5	—	—
	1.5	10	—	—
	1.5	5	—	—
	1	10	—	—
	1	5	—	—
Spores grown on wheat agar.......	2	10	—	—
	2	5	—	—
	1.5	10	—	—
	1.5	5	—	—
	1	10	—	—
	1	5	—	—

— = No growth.

ture No. 1 showed that the neutralization and washing would not remove or destroy the spores. All samples of acid-salt treated skin remained sterile in both bouillon and dextrose-serum-bouillon (see Table V).

TABLE V.

Final Schattenfroh test.

Culture No.	Source.	Grown on	Inoculation.	HCl %.	NaCl %.	Time of Exposure.	Neutralization.	Wash.	Subculture in	
									Bouillon.	Dextrose Serum Bouillon.
1	Laboratory stock	Wheat agar 3 days	Surface and Intradermal	2	10	42 hrs.	4% Soda ½ hr.	Until neutral	—	—
3	Tannery vat	"							—	—
4	"	"							—	—
43	Horse hair	"							—	—
1	Laboratory stock	"	"	— Control.	—	—	"	"	+	+

+ = Growth.
— = No growth.

HORSE HAIR DISINFECTION TESTS.

As part of the investigation of the horse hair dressing industry additional tests of methods of killing anthrax spores were carried out and have already been reported (12), but will be described again. The problem of disinfection of hair is much simpler than that for hides, as the hair is less easily damaged and the spores are less protected by organic matter. The Schattenfroh and Seymour-Jones methods are rather expensive for use with hair and require the handling of dangerous substances. Steam under pressure in the autoclave will easily and simply kill all anthrax spores in hair, but some hair workers claim that it injures the hair. The Bureau of Animal Industry regulations (4) permit the so-called "disinfection" of horse hair for anthrax by exposure to 200° F. for 15 minutes, but the Bureau Inspectors insist that this exposure shall be for 24 hours, and shall be preceded by washing in hot alkaline suds. As carried out in Philadelphia the usual procedure is to soak over night in a strong hot soda solution, scrub on a wash board with a hot soap solution and then dry in a closed room for at least 24 hours. The room is usually heated by a red hot coal stove and is a very decided fire risk with the racks of horse hair all around the stove.

HEAT TESTS.

As was expected, tests of the efficacy of dry heat showed that 15 minutes' exposure to 200° F. had little if any action on anthrax spores, but in every instance a 24-hour exposure at this temperature killed all bacteria and spores on hair inoculated with anthrax. Table VI gives the results of the heat tests referred to, including under *A* the results of autoclaving, under *B* of dry heat alone and under *C* of heat preceded by washing, as described above. In the last column of *C* it will be noted that only one anthrax colony developed after a 48-hour exposure to 175° F. in one of three tests. Subsequent tests at 200° F. gave the same results as in *B*, that is no colonies developing after a 24-hour exposure. The effect of dry heat at this temperature was tried on anthrax infested skin, but it was found to be uncertain even after a 48-hour exposure, and the skin was injured so it could not be satisfactorily tanned.

CYLLIN TESTS.

A sample of Cyllin was secured from a curled hair manufacturer who had imported it from England, and tests were carried out as

TABLE VI.

A. *Disinfection tests for anthrax on horse hair.*

	Unsterilized.	Autoclaved.
Undressed loose hair.......	10 short hairs plated in agar gave innumerable colonies.	15 lbs. for 30 minutes; 10 hairs gave six colonies.
Dressed hair bundle (6 in. long; 3 in. thick at butt)..	4 hairs plated in agar gave innumerable colonies.	4 hairs gave 32 colonies.
Above sterilized bundle inoculated through center with 5 cc. anthrax broth..	4 hairs gave innumerable anthrax colonies.	No anthrax colonies in any of 12 plates containing 4 to 8 hairs.

B. *Dry Heat 200° F. (93° + C.).*

	15 min.	30 min.	1 hour	2 hours	24 hours	48 hours
Sterile undressed hair infected with anthrax broth..............	4 hairs abundant growth	4 hairs abundant growth	4 hairs abundant growth	4 hairs abundant growth	10 hairs no growth	50 hairs no growth

C.

Sterile undressed hair infected with anthrax broth	Hot Na_2CO_3 10% 18 hours	Scrubbed in hot soap sol. 10 min. and rinsed in hot water	175° F. (79° + C.)	
			24 hours	48 hours
Sample 1.......	4 hairs in agar numerous anthrax colonies	4 hairs in agar no anthrax	4 hairs no anthrax	50 hairs 1 anthrax colony
Sample 2.......	4 hairs in agar 2 anthrax colonies	4 hairs in agar no anthrax	4 hairs no anthrax	No anthrax
Sample 3.......	4 hairs in agar no anthrax	4 hairs in agar no anthrax	4 hairs no anthrax	No anthrax
	Broth culture from agar plate of sample No. 1 killed guinea pig in 3 days.	Agar and broth cultures from soap solution after washing showed no anthrax.		

follows: A mass of loose horse hair trimmings was sterilized in a beaker in the autoclave, covered with a heavy suspension of anthrax spores, let stand 2 hours at room temperature, drained and allowed to stand 2 days covered to dry and then covered with a 2 per cent. Cyllin solution, the strength used in England. The hair was thoroughly worked with a glass rod after covering with Cyllin to remove any air locks from included bubbles. The earlier certificate given in England (1908) called for a one-hour exposure at 60° F. in a 1 per cent. solution, but this was altered in 1912 to require a two-hour exposure at 98° F. in a 2 per cent. solution. Three tests were run with Cyllin 2 per cent. solution, one at 60° F. and two at 98° F. At hourly intervals samples were removed, washed in water and transferred to bouillon and dextrose-serum-bouillon. Table VII gives the results of these tests and shows that the 2 per cent. solution failed to kill anthrax in 4 days at 98° F. This agrees with information furnished the author by Dr. T. M. Legg, Chief Medical Inspector of Factories for Great Britain, in which he stated that "anthrax was cultivated after 28 days' exposure."

TABLE VII.

Disinfectant action of cyllin on anthrax.

Horse hair infected with mixed anthrax spore suspension and covered with 2 per cent. cyllin solution.

Temp. of test	Subculture after exposure for					
	1 hour	2 hours	3 hours	5 hours	6 hours	4 days
60° F.......	+	+	+			
98° F.......	+	+	+			
98° F.......	+	+	+	+	+	− bouillon + dextrose-serum bouillon

+ = Growth.

IDENTIFICATION OF ANTHRAX COLONIES.

In endeavoring to isolate anthrax organisms from cultures from horse hair the suggestions of Hagan (13) as to the identification of anthrax by microscopic examination of the colonies formed on agar were taken advantage of. By this method laboratory cultures indistinctly labeled were identified as anthrax, and in an examination of 83 series of plates in all with more or less curled colonies, 14 plates were found to show typical anthrax-like colonies. These plates represented six strains, five from imported China hair and one from Canadian hair, but only one of these strains succeeded in killing guinea-pigs. Hagan states that true anthrax colonies on + 1.5 per cent. agar, when examined under the oil immersion lens, show very marked curling, with long regularly parallel threads, showing dividing lines between cells with difficulty or not at all, with few free ends of threads visible, not showing spores in 24 hours and showing no motile cells. Using this method of differentiation the author has identified five organisms virulent for guinea-pigs, and five others which failed to kill pigs or white mice. Possibly these were avirulent anthrax, but the author prefers to call them pseudo-anthrax and to depend on animal inoculation alone for final diagnosis using Hagan's method as a simple means of excluding more or less closely related soil organisms usually found in suspected materials. The author has the same views as to the value of deep colony peculiarities in the identification of anthrax.

Jackson and Buswell in the Journal of the American Leather Chemists Association (14) report a series of experiments on the disinfection of tannery washes in which they recommended the use of liquid chlorine 50 parts per million to disinfect tannery effluents though he states that it would take from 160–200 parts per million to thoroughly sterilize. On account of the great affinity of the chlorine for other organic matter neither chlorine nor bleach were tested by the author. The English authorities, I am informed by Dr. T. M. Legge, are contemplating using the same method for disinfecting horse hair that has been elaborated there for the disinfection of wool; namely the use of a hot alkaline soap bath with agitation, squeezing through rollers, agitating in a warm formaldehyde solution, squeezing and drying (15), but this was not tested by the author as it requires rather elaborate apparatus and considerable manipulation.

Summary and conclusions.

This paper gives details of several series of tests carried out by the author on the disinfection of skins and horse hair for anthrax.

In these tests five strains of anthrax bacilli were employed, an old laboratory stock strain, three strains isolated from tannery soak vats and one strain isolated from horse hair.

Especially resistant spores were obtained by preliminary growth on wheat agar.

Rejuvenation of spores damaged by treatment, but not killed was encouraged by subculture in dextrose-serum-bouillon.

In testing action on skins the most satisfactory method of preparing test samples was found to be intradermal inoculation of soaked squares of skin with saline suspensions of anthrax spores.

The lime soak of 5 per cent. CaO permitted by the Bureau of

Animal Industry regulations proved to be unreliable, even after a 72-hour exposure.

The Schattenfroh method of soaking skins in 2 per cent. HCl and 10 per cent. NaCl solution for 48 hours at room temperature gave uniformly good results if carried out under conditions similar to those employed in the tannery, and produced skins that could be made into satisfactory leather.

Anthrax infested horse hair can be satisfactorily disinfected by several methods:

(*a*) By steam under pressure in the autoclave (15 lbs.) for 30 minutes.
(*b*) By dry heat—200° F. for 24 hours (but not 200° F. for 15 min. as permitted by the Bureau of Animal Industry).
(*c*) By formaldehyde as used in England.

Cyllin disinfectant in the strength and under the conditions certified in England will not kill anthrax spores.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE.*

I. GENERAL INTRODUCTION.

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A. Factors involved in hookworm control.

The gradual accumulation of information on the life cycle of the hookworm has made it possible to apply control measures with increasing precision. Much has already been accomplished, and the experience of men engaged in control work is constantly pointing to problems, the solution of which will make the attack on hookworm disease more effective. It is the purpose of this first article of the series to analyze the factors involved in hookworm control and to suggest certain lines which need further investigation.

To control a disease produced by a parasitic organism it is necessary to find a point at which its life-cycle can be broken. The life-cycle of the hookworm including as it does both a parasitic stage and an active free-living stage offers three points of attack, viz., the parasitic adults, the eggs and the newly hatched larvae, and the infective larvae. As applied to control measures the attempt to kill the parasitic stage of the hookworm includes (1) the recognition, usually by fecal examination, of the individuals who harbor the parasites, (2) the administration of an anthelminthic to infested individuals, (3) the reexamination after treatment to discover if the worms have been dislodged, and (4) the retreatment, until the examination becomes negative, of those individuals who are still infested. The killing of the hookworm eggs can be accomplished immediately by disinfection of the feces containing them, or by the prevention of the deposition of feces by infested individuals under conditions where the eggs can hatch and reach the infective stage. This phase of the attack centers around the prevention of soil pollution, and is also effective in the control of other diseases, such as typhoid fever and the protozoan and bacillary dysenteries. The killing of the infective hookworm larvae may be accomplished directly by disinfecting the soil in which they live, or indirectly by preventing the human host from coming into contact with such soil under conditions which would make infestation possible.

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* The series of papers which will be published under this title represents the results of hookworm investigations carried out by the Department of Medical Zoology of the School of Hygiene and Public Health of Johns Hopkins University with the cooperation of the International Health Board of the Rockefeller Foundation.

It is impossible to outline any standard method of hookworm control which would be applicable under all circumstances since the conditions vary so greatly. It is possible, however, by critical analysis to evaluate in a very general way the measures of control commonly used. First, it must be constantly borne in mind that in a campaign for the control of hookworm disease the object is primarily to kill worms and the results must in the main be judged not so much by the number of patients cured as by the reduction of the total infestation of the region where the work is being done. That the reduction of mass infestation is not necessarily synonymous with patients cured was brought forcibly to my attention several years ago in connection with the hookworm control work in the mines of California. In a mine, slightly out from the endemic center, examinations showed twenty positives out of about one hundred underground workers. All except two of these infestations were very light, showing by the culture method of diagnosis only one or very few larvae from a culture. Almost all of these cases would have been negative if one of the more usual methods of fecal examination had been used. The other two men had extremely heavy infestations which they had evidently acquired in a heavily infested mine in which both had worked three years previously. It was roughly estimated that these two men harbored at least ten times as many worms as all of the other eighteen together. In the report the twenty cases were marked positive and looked much alike, but for the control of the disease in the mine the two heavily infested individuals were much more important than all of the other eighteen put together. Especially was this true since one of them had habitually defecated in a sump which was visited by the other workers and which was literally swarming with hookworm larvae.

A second general consideration in connection with hookworm work is that each control project differs from every other and therefore must be attacked as an independent problem. The factors which must be considered in determining the best method of procedure in any given case are so complex that the director of the campaign must not only investigate the conditions carefully before planning his work, but must be constantly alert to vary his methods as new information makes it seem desirable. The best methods to be used in any given hookworm campaign depend among other things on the purpose of the work, the resources available, the character and customs of the people, the conditions under which they live, the degree of infestation and the chief sources of infestation. For example, if a plantation owner with a shifting labor population wishes to increase the efficiency of his labor, he will be interested chiefly in the reduction of mass infestation in his workers, and will pay special attention to the sources of infestation on his plantation. A medical man working alone with a small appropriation would hardly concern himself with the improvement of his method of examination or with completely curing his patients, but would try to treat the worst cases and spread as much information as possible. Where human excrement is used for fertilizing the soil the methods of attack become quite different from a region in which this practice does not exist. The hookworm campaigns carried on under army conditions assumed an entirely different aspect from those in a civil population, because it was possible to absolutely control the individuals with whom the work was being done. Examples of this kind could be multiplied, but those given serve to illustrate the futility of trying to work out by rule of thumb any best program of hookworm control.

In the inauguration of a hookworm campaign the discovery of the exact sources of infestation is of great importance. Without this information the control measures in a region can only be directed at a general reduction of mass infestation. The importance of developing methods for making definite epidemiological studies on hookworm disease is emphasized by the helplessness many workers feel when they find a high degree of reinfestation of the population of a region only a short time after the conclusion of an intensive hookworm campaign. That it is feasible in connection with a hookworm campaign to study intensively the sources of infestation is well illustrated by the work of Baermann (1917)[1] in Sumatra. This investigator has worked out a simple method by which infective hookworm larvae can be isolated from soil (Baermann, 1917).[2] By the use of this method he has apparently been able to uncover the chief sources of infestation in the area in which he was working. He was led to make these studies by the fact that even after applying the ordinary measures for a period of several years there was a considerable degree of infestation which he was unable to eradicate. I will not go into the many interesting details of his work, but will only mention his finding of one entirely unsuspected source of infestation. He found hookworm larvae in large numbers in damp areas on the dirt floors of the houses around the places where the washing and drinking water was kept. He suggests a number of ways by which the larvae might have been brought to these places, and concludes that these spots were the chief source of infestation for the people living in the houses. Here then was an important source of infestation the detection of which at the beginning of the work would have made the whole campaign more effective.

In analyzing the factors involved in hookworm control the phases of the work connected with the killing of the parasitic stage, or in other words, the treatment, will be first discussed. Before treating, examinations are usually made to find the individuals carrying the infestation. Certain workers have advocated the omission of the diagnostic examinations and the treatment of the whole population where a preliminary survey has shown a very high mass infestation. Such a procedure might be justifiable where over eighty per cent. of the population are infested and would result in a considerable saving. The factors involved in the choice of any particular method of fecal examination for the diagnosis of hookworm disease are so complex that it is not possible to say that any given method is the best for all circumstances. The choice of method must depend on the purpose of the work, the number of examinations to be made, the money available, the conditions under which the work is to be done, the qualifications of the microscopists, etc. It is practically impossible to make the diagnostic examination so critical that every individual who harbors hookworms will be marked positive. Also, in a certain number of the cases the number of worms present is so small that such individuals could hardly be considered an appreciable factor in the spreading of the disease. The method of examination used should certainly be sufficiently accurate to find all those who harbor a sufficient number of worms to produce clinical symptoms, as well as those who have a considerable degree of infestation but high resistance. In most campaigns the accuracy of the examination can be carried far beyond this point, but it should not be carried to the extreme where the increased cost in time and money is out of proportion to the results obtained.

In the treatment phase of the work quite a proportion of the time and money expended often goes to the attempt to ''cure'' the patients treated. Sometimes four or five treatments and the corresponding reexaminations are given a single patient before he is pronounced ''cured,'' the term ''cured'' being applied when the number of worms has been reduced below the point where the method of examination in use gives a positive finding. It is evident that where less critical methods of reexamination after treatment are used there will be a larger number of ''cures.'' On account of this possible source of error and the time consumed there has been a tendency recently to question the value of this phase of the work. Perhaps further experiments will make it possible to standardize so definitely the methods of treatment for hookworm disease that all reexaminations and subsequent retreatments will become unnecessary. Certainly the expenditure of considerable portions of moneys available for hookworm control work in attempts to clear out the last few worms from the patients is not justifiable when we consider the millions suffering from hookworm disease who cannot be reached by any work now organized.

In the attempt to break the life-cycle of the hookworm by killing the eggs, control work has been centered around the prevention of soil pollution. This phase usually consists in seeing that the people are provided with proper latrines and are educated to use them. In many ways this is the easiest and most effective measure for hookworm control and without its inclusion no campaign can have lasting value. It has the advantage of being also an attack on the numerous other diseases which are spread by soil pollution. In this phase of the work there are two critical points. First, it must be certain that the type of fecal disposal which is advocated really prevents the spread of the disease. Second, the whole success of this phase of the work rests on whether the people of the area will be sufficiently enlightened to cause an appreciable number of them to continue to maintain and use the latrines after the conclusion of the campaign. Unfortunately in many areas where hookworm work has been carried on there has not been any great decrease in soil pollution, and time after time even where proper latrines are installed in adequate numbers only a short time elapses before the people have returned to their former habits of soil pollution. In regions where the population is very primitive or where human excrement is used for fertilizing the soil the problem seems almost incapable of solution. The habits of people are not easily changed. Only by educational activities carried out by governmental agencies over a term of many years and supplemented by carefully planned and adequately enforced legislation can it be hoped to permanently improve in many places the methods of excreta disposal. To prevent pessimism in regard to present hookworm control campaigns it should be remembered that in many cases they are merely entering wedges designed to start in motion those larger agencies that may in time be able to raise the standards of the people.

Connected with the prevention of soil pollution in the general educational campaign is the question of the prevention of the entrance of the infective larvae into man. The manifold phases of this need not be discussed here, but it is necessary to throw out the caution that since in most areas the actual sources of human infestation are not known, it is only when careful epidemiological investigations are carried out that information of more than a very general nature can be given to the people for their personal protection.

There has been considerable discussion and much experimentation on possible methods of killing hookworm eggs and larvae in the soil. As compared with bacteria, the infective hookworm larvae are surprisingly resistant to disinfectants. In considering the effect of various chemical reagents on hookworms in the soil it must be remembered that all stages of the hookworm in the soil are not affected in the same way. Experiments have shown that any given chemical agent which might be used for disinfection acts differently on the eggs, the newly hatched larvae and the infective larvae. The newly hatched larvae are much more easily killed than either of the other stages. It is apparently not practicable to disinfect generalized sources of infestation. It is possible, however, that this method of hookworm control has a much more important application than has been realized. Certainly it is possible to disinfect limited areas such as highly polluted mine "sumps," the areas around faulty latrines, or infested places in a house or yard. Unfortunately our knowledge is still unsatisfactory in regard to the best disinfectants to use and their method of application.

B. PROBLEMS IN HOOKWORM CONTROL.

There is available a large body of information both on the life-history of the hookworm and the methods of hookworm control. There is, however, no stage of the life-cycle of the hookworm or no phase of hookworm control work on which further investigations cannot be carried out with profit either from the biological or from the practical point of view. The problems which need investigation in connection with the improvement of hookworm control work can for the sake of convenience be divided into two general categories. The first involves researches on methods of control and the second researches on the biology of the hookworm.

Researches of the first type have for their purpose the reduction in the cost of the methods used or the increase of their effectiveness. When the large sums of money which are being spent in hookworm control and the vastness of the field yet untouched are considered it can be realized how important is any saving or improvement in method, however slight. Researches on improvement of methods can usually be best carried on by men connected with active control work in the field, and from such men results are constantly coming which have added much to the effectiveness of hookworm control work. The director of a hookworm campaign who adequately grasps the significance of his project as an individual research problem can hardly fail to add materially to the improvement of the methods of work. The differences of opinion which have arisen show that there is still plenty of room for further investigations of this type. For example, much can be expected from further comparative studies of the methods of fecal examination and their adaptation to varying conditions. In spite of all that has been done, the treatment of hookworm disease still needs investigation, and progress can be expected both from the comparison and improvement of methods now in use and from the search for new ones. The further development of methods for the disposal of human excreta which can be applied to various regions where soil pollution is prevalent is a promising field. Along this line the U. S. Public Health Service is devoting large sums of money and the services of a number of experts in an attempt to devise methods for the disposal of human excreta which will be adapted to conditions in the Southern United States. Little that is satisfactory is yet known about the disinfection of the soil. In fact every step in hookworm control work still needs to be carefully investigated from a variety of different viewpoints and under different conditions.

In spite of the great mass of published data which is available on the life cycle of the hookworm, it still offers many unsolved problems. While any new information on any phase of the biology of the hookworm may prove of practical value in the attack on the organism, it is that phase of the life-cycle which is passed outside of the human body which offers the most promising field. Since the investigations which we are undertaking center around the life of the hookworm eggs and larvae in the soil a more extended analysis of the unsolved problems of this stage and their practical applications will be made.

From the biological point of view the investigation of the life of the hookworm in the soil is primarily an ecological problem involving as it does a complete study of the influence of all the various factors of the environment on this phase of the life of the organism. From the point of view of control this problem centers around the question of the infectivity of the soil. It involves a study of the distribution of the hookworm eggs and larvae outside the human body, especially their distribution to situations where they will have a chance to reenter the human body, and of measures which could be employed to keep them from such situations. Such investigations include those which have to do with the attempt to increase knowledge concerning the general relation to environment and activities of this phase of the life-cycle of the hookworm and those which are centered around the problems of the source of human infestation in particular areas. There is really very little precise information on the life of the hookworm in the soil, and seldom has the infectivity of the soil in any given area been scientifically studied. The importance of such studies is apparent when we consider, as was shown earlier, that unless the exact sources of infestation of the people in a given area are known, only general control measures can be applied.

In order to more clearly define the unsolved problems in connection with the life of the hookworm in the soil I will present in outline the phases which need investigation and discuss the practical applications of some of the problems suggested.

Certain phases of an investigation of hookworm in the soil.

I. The eggs and young larvae.
  A. The distribution of the eggs over the soil.
    1. By man.
      a. By direct soil pollution.
      b. By accidental carriage on feet and hands.
      c. By use of human excreta in fertilizing the soil.

- 2. By animals other than man.
    - *a*. By feeding on human feces.
    - *b*. By human feces clinging to parts of the body.
- 3. By physical agencies.
    - *a*. By rain.
    - *b*. By bodies of water.

B. Conditions necessary for the development of the hookworm in the soil.

- 1. Length of time under varying conditions that the hookworm eggs will remain alive without hatching in human stools.
    - *a*. In stools on the soil.
    - *b*. In stools in water.
    - *c*. In stools in various types of latrines.
    - *d*. In buried feces.
- 2. Hatching of eggs and development of larvae under various environmental conditions.
    - *a*. Will hookworm eggs hatch in buried feces?
    - *b*. Hatching and development in various types of latrines.
    - *c*. Conditions necessary for hatching and development when deposited on the soil or in water.
        - (1) Food.
        - (2) Moisture.
        - (3) Temperature.
        - (4) Chemical conditions.

II. The infective larvae.

A. Length of life.

- 1. Under conditions favorable for great activity.
- 2. Under conditions which favor inactivity.
- 3. In various types of soils.
- 4. Length of time various soils remain infective after pollution stops.

B. Conditions necessary for life.

- 1. Amount of moisture needed.
    - *a*. Effect of large amounts of moisture.
    - *b*. Resistance to desiccation.
- 2. Chemical relations.
    - *a*. Favorable and unfavorable chemical situations in natural environment.

        b. Effect of various chemical agents.
        c. Disinfection of infested soil.
- *C*. The spread of the infective larvae.
    1. Active migrations.
        - *a*. Horizontal migrations from pollution centers.
            - (1) Effect of temperature.
            - (2) Effect of moisture conditions.
            - (3) Effect of physical character of the soil.
            - (4) Effect of chemical character of the soil.
            - (5) Effect of vegetation.
        - *b*. Vertical migrations.
            - (1) Depth of vertical migrations under favorable conditions.
            - (2) Possible upward migration when larvae are buried.
            - (3) Possible downward migration when upper surface of soil dries.
    2. Passive spread of infective larvae.
        - *a*. By man.
        - *b*. By animals other than man.
        - *c*. By physical agencies.
            - (1) By rain.
            - (2) By streams of water.
    3. The distribution of infective larvae in an infested area.
        - *a*. With respect to centers of soil pollution.
        - *b*. In relation to disposal of human wastes.
        - *c*. With regard to sources of human infestation.

I shall not attempt to discuss all of the problems which are suggested by this outline, but will merely indicate the importance of the solution of some of them. In the epidemiological study of any given area, the study of the centers of soil pollution and the analysis of the factors involved in the spread of the feces containing eggs would naturally be the first step. In certain regions chickens, dogs, and pigs ingest human feces in considerable quantity. While the rôle of these animals in the spread of hookworm disease has been suggested frequently, their real significance has not been sufficiently investigated. A more accurate understanding of whether these and other animals can spread ingested hookworm eggs or larvae would make it possible to determine whether their activities in any given region were producing definite centers from which the people were being infested.

After an analysis of the spread of the feces containing eggs would come the study to determine just where the eggs find conditions favorable for their development and hatching. Unfortunately, our information is not very clear on a number of factors involved in this phase. Several insufficiently answered questions center around the question of how long hookworm eggs live when they are deposited under conditions unfavorable for their development. The fact has been established that hookworm eggs can remain viable for considerable periods when conditions are unfavorable and then when conditions become favorable can continue their development. It is probable that this power of resistance is partly responsible for the much greater incidence of hookworm than of Strongyloides stercoralis where these two organisms live side by side. To evaluate this factor in any given situation more information is needed to answer such questions as the following: How long can hookworm eggs resist desiccation in a stool deposited on the surface of the soil? How long can eggs remain capable of hatching under conditions such as those within a solid stool or a buried stool in which the supply of oxygen is not favorable for development? A very practical question in this connection is whether the eggs remain viable for any considerable time and especially whether they can hatch and the larvae develop under the conditions found in various types of latrines. Also, whether larvae which have developed in latrines can escape to situations where they would be dangerous to man. Many more investigations are needed on the factors involved in the hatching of the hookworm eggs and the development of the larvae to determine the conditions necessary for such development under natural conditions.

The literature contains a number of references to observations on the length of life of hookworm larvae in the infective stage. In this connection the particular point which needs further investigation is how long after the removal of the sources of soil pollution can a given area remain infective. That is, how long do larvae live under various situations in such numbers as to be an appreciable source of infestation to the population. Since the infective hookworm larvae depend for their food and oxygen on stored material, it seems very probable that the more active they are, the sooner they will die.

We know very little of the wide variety of factors which influence the life of the hookworm larvae in the soil, and further studies are needed on such questions as resistance to chemicals, soil preference, etc. The evidence indicates that desiccation quickly kills the hookworm larvae. An important series of investigations which are greatly needed are those which would determine the minimum of moisture which must remain in the soil of various types to keep the hookworm larvae alive. Further, can hookworm larvae migrate down into the lower layers of the soil as the upper layers become dry? Both of these problems touch the practical question of whether hookworm larvae can live over the dry season in certain tropical regions.

There are few other questions on the biology of the hookworm on which we have less accurate data than on the migrations of the infective larvae. It is not so much, how far theoretically they might migrate, as how far they actually do spread under various conditions from the centers of soil pollution. Studies under both laboratory and field conditions are needed along this line, involving such questions as the influence of the physical and chemical character of the soil and its moisture content on such migrations.

Most of the problems which have just been discussed are related to the question of the sources of human infestation. Such problems are not easily solved, and many of them depend on the development of methods which make it possible to discover whether encapsuled hookworm larvae are present in the soil. On this point Baermann (1917)[2] has been most successful by utilizing the fact that the larvae will leave soil for water. In the work which we are undertaking on some of these problems a modification of Baermann's apparatus has been used for isolating infective hookworm larvae from the soil, and a description of it will appear in number two of this series. Such an analysis might be carried much further, but it has been sufficient to define the problems of the present series of investigations, and possibly to suggest to other workers lines of research which might be followed with profit.

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FIVE SPECIES OF TSUTSUGAMUSHI (THE CARRIER OF JAPANESE RIVER FEVER) AND THEIR RELATION TO THE TSUTSUGAMUSHI DISEASE.*

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PLATES XXXIV TO XLI.

INTRODUCTION.

It is a long-established fact, that the tsutsugamushi disease or Japanese river fever is transmitted to man only by the bite of a mite, the tsutsugamushi (*Trombicula akamushi* (Brumpt)). The life cycle of the mite was clearly demonstrated in 1916 by the authors (1, 2) and shortly afterwards by Kawamura (3) and Miyajima (4, 5) and their co-workers, whereby its biological and morphological characteristics were completely ascertained in every stage of development. The life cycle of tsutsugamushi is characterized by three separate stages, a mature, nymphal and larval stage, with a pupal stage between each two, as in most other acari. Only in the larval stage does the mite instinctively attack some warm-blooded animal in order to derive nourishment for its further development. As its principal host in this period it commonly seeks voles to feed upon.

Though in all the endemic districts voles are found throughout the year carrying the larvae of the mite on their ear-lobes, man is never attacked by them except in seasons from June to October. It has been supposed, with regard to this fact, that the mites are too tardy in their motility in cold weather to attack man, or that field laborers rarely need to enter infected places except in summer, so that very few are bitten by mites. This explanation is as yet unconvincing, because warm-blooded animals brought into these districts are sure to be attacked by them in any season although man is never bitten under any circumstances in the same place and at the same time.

Tanaka (6), however, was the first to insist upon the existence of two forms of the mites which are usually termed tsutsugamushi or akamushi in endemic districts. According to his many years' experience he ascertained that the mite which attacks the human body is always of unique form, though on the ear-lobes of voles in these places another one is found besides this, which differs in a morphological point of view from the former, and he designated this species as Yasodani (vole mite), presuming it to be a different species. Later Miyajima and Okumura (7) admitted these two forms with a more detailed morphological description, but they thought these changes to be only seasonal variations of the same species.

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According to our investigations since 1918 on this subject, we were able to confirm in 1919 (8) three forms of tsutsugamushi, two of which have been described by Tanaka and Miyajima, while the other has not yet been observed by other investigators. We assumed it both from a morphological and biological point of view to be a third form of tsutsugamushi, which appears abundantly except in summer, and might be the one confused with the tsutsugamushi proper on some occasions. Our researches on this problem in successive years (9, 10) threw much light upon the morphology as well as the biology of these mites, and we are now able to state positively that there are up to the present at least five species of tsutsugamushi which differ not only morphologically, but also biologically from each other, and show different seasonal prevalence, and among these only one is responsible for the occurrence of the human tsutsugamushi disease.

DESIGNATION AND SYNONYMY OF FIVE SPECIES OF TSUTSUGAMUSHI.

1. *Trombicula akamushi* (Brumpt) 1910.

Kedani Tanaka's; thin-haired type after Miyajima and Okumura; Kawamura's type *A* (11).

2. *Trombicula pallida* n. sp. 1919.

Yasodani (vole mite) or Pseudo-akamushi Tanaka's; coarse haired type after Miyajima and Okumura; Kawamura's type *D*.

3. *Trombicula palpalis* n. sp. 1919.

Kawamura's type *C*.

4. *Trombicula intermedia* n. sp. 1920.

5. *Trombicula scutellaris* n. sp. 1920.

Kawamura's type *B*.

MORPHOLOGY OF EACH SPECIES.

Larva.

Up to the present we have hatched the larvae of *Tr. akamushi, pallida* and *palpalis* from their adults artificially in bottles. These newly hatched larvae as well as those of *Tr. intermedia* and *scutellaris* are almost the same in size. They measure, whilst unfed, 0.212–0.264 mm., 0.244 mm. on an average from the tip of the chelicera to the posterior margin of the body, and 0.128–0.172 mm., on an average 0.145 mm. in width. When gorged they increase remarkably in size and may attain to twice the original or even more. The body is in all species ovoid in form, at times somewhat narrower in front in unfed or partially fed specimens, but after sucking enough on warm-blooded animals they become more elliptical.

In the larval stage they are hexapod, each leg consisting of six articles, and the tarsi are all provided with three claws. The length of the legs is in respective pairs almost the same in each species, the first measuring 0.185–0.19 mm., the second 0.16 mm. and the third 0.2 mm. on an average. Also in the form and size of the claws they do not differ from each other.

The hairs on the legs may be divided into four forms, i.e., feathered hairs, bristles, rod-shaped setae and spines. They are arranged in respective pairs of legs entirely in the same manner in all species as is shown in the following table, a fact which may indicate close relationship of the five species to each other.

TABLE I.

Leg.	I.						II.						III.					
	Article.						Article.						Article					
Hair.	I.	II.	III.	IV.	V.	VI.	I.	II.	III.	IV.	V.	VI.	I.	II.	III.	IV.	V.	VI.
Feathered hairs	1	1	5	4	8	21	1	2	4	3	6	16	1	2	3	3	6	15
Bristles				2	2	3				1	2	2				1	1	
Rod-shaped setae						1						1						
Spines				1	1	1						1						

These feathered hairs of the leg, having two rows of plumes on their convex side, resemble rather those on the ventral surface of the body of the larva, but they are much thinner than the latter.

Epimeres, tracheal opening, maxilla and mandible, double eyes with lenses on both sides of dorsal shield, anus, joints of palpus and palpal claw, etc., show no remarkable difference among the five species.

The above description applies to each species and may be the general characteristics of the tsutsugamushi in differentiating them from other acari.

On the other hand there are fixed morphological differences in each species in the character of the hairs of the body and palpus, and those on the dorsal shield and the form of the latter. We could never detect transition forms between the species in any season of the year.

1. *Hairs of the body.*

The tsutsugamushi mites, which bear the name Kedani (hairy mite), are provided with many hairs on the ventral as well as the dorsal surface of the abdomen. In general the hair bends more or less to one side, in the natural position of the larva, the concavity facing the surface of the body. The hair is more or less pinnated in four rows, two on the convex side and the rest on the other. The pinnules on the convex side are two or three times more in number than those of the other. The character of the lateral branches and the angle between them and the trunk of the hair differ more or less according to the species. These specific characteristics are most prominent in the hairs on the dorsum of the larvae, and particulars of it may be tabulated as follows (Table II):

The hairs on the ventral surface of the body, pinnated in two rows on their convex side, show very little difference among these species, except in number. It is worth noticing that in *Tr. palpalis* the solitary hair on coxa III is situated on the anterior border of the latter, which is not the case in other species.

2. *Hairs on palpus.*

With the exception of the hairs on the third article of the palpus we have not yet detected any difference among the species. Articles I and II are each provided with one plane hair on their dorsal surface. On article III there are three bristles, one each on the dorsal, lateral and ventral surface respectively. In *Tr. palpalis,* however, the latter constantly bears 4–5 lateral branches, the only and definite difference in the morphology of the palpus of *Tr. palpalis* as compared with the other four.

TABLE II.

Hairs on dorsum / Species	Character of lateral branches	Number of lateral branches on convex side, in each row	Number of lateral branches on concave side, in each row	Total number of hairs on dorsum, except those on scutum (on an average)	Number of dorsal hairs in the first row	Length of hairs in the first row (on an average)
Tr. akamushi	delicate, indistinct in low magnification	12–13	6	37+	commonly 10	48.0 μ
Tr. pallida..	thick and long, at the tip obtuse, distinctly remarkable in low magnification	10–11	4	60	commonly 14	43.3 μ
Tr. palpalis.	middle sized, not so long as in *pallida*, pointed at the end	11–12	5	45	commonly 13	51.4 μ
Tr. intermedia....	thick, but less than in *pallida*; tip obtuse	15 and 17 or 14 and 17	4 and 5 or 3 and 5	38	commonly 12	57.5 μ
Tr. scutellaris......	similar to that of *palpalis*, somewhat more slender	10–11	6	51	commonly 12 or 13	51.2 μ

3. *Dorsal shield and hairs upon it.*

The form of scutum and hairs upon it and the manner of arrangement of the latter are the most remarkable points for the morphological differentiation of the five species from each other. Each has one dorsal shield, generally rectangular in form and about the same in size, measuring sagitally 37.7 microns and 71.7 microns in width on an average, except *Tr. scutellaris,* in which the average width of the dorsal shield attains to 82.3 microns. In each species there are five pinnated hairs and a pair of sensory hairs, which bear distinct specific characteristics as is seen in the following table.

TABLE III.

	Form of scutum	Pinnated hairs on scutum	Position of posterior lateral hairs on scutum	Sensory hairs
Tr. akamushi...	rectangular; posterior angles not rounded	similar to those on dorsum	laterally and about 9 μ posteriorly to sensory hairs (pseudostigmatal organ)	length 64.8 μ; proximal part naked; ratio of proximal and distal parts about 2 : 3
Tr. pallida.....	rectangular; posterior angles slightly rounded	do.	laterally and about 5–6 μ anteriorly to sensory hairs	length 64.8 μ; proximal part with minute spines; ratio of proximal and distal parts about 1 : 2
Tr. palpalis....	rectangular; posterior angles distinctly rounded	do.	laterally and anteriorly to sensory hairs	length 61.4 μ; proximal part naked; ratio of proximal and distal parts about 1 : 3
Tr. intermedia..	rectangular; posterior angles distinctly rounded	do.	laterally and anteriorly to sensory hairs	length 72.0 μ; proximal part with minute spines; ratio of proximal and distal parts about 1 : 2
Tr. scutellaris ..	rectangular; posterior angles distinctly rounded	do.	laterally in the same height with sensory hairs	length 77.1 μ; proximal part with trace of spines; ratio of proximal and distal parts about 3 : 5

BREEDING EXPERIMENTS.

Up to the present we have been able to confirm the life cycle of the mites in vitro, thus:

1. *Tr. akamushi*
   - *a.* Larva (from voles) → Nymphophane → Nymph → Teleiophane → Prosopon
   - *b.* Prosopon (captured) → Larva
2. *Tr. pallida*
   - *a.* Larva (from voles) → Nymphophane → Nymph → Teleiophane → Prosopon

  b. Prosopon (captured) → Larva

3. *Tr. palpalis*
  a. Larva (from voles) → Nymphophane → Nymph → Teleiophane → Prosopon
  b. Prosopon (captured) → Larva
4. *Tr. intermedia*
  Larva (from voles) → Nymphophane
5. *Tr. scutellaris*
  Larva (from voles) → Nymphophane → Nymph

In these experiments dried soil, taken from the endemic district in Yamagata Prefecture several years ago, and undoubtedly containing no living mite, was put into culture bottles. Before laying the larvae or adults therein the soil is moistened with a proper quantity of water. The breeding was carried on at room temperature and in incubators at 22° and 30° C. In the course from nymph to adult it is necessary to put into the bottles some vegetable debris as food for the nymphal stage, otherwise the emergence of mature animals is not usually to be expected. Before putting into bottles the larvae must be assorted without mounting under the cover-glass, and this may be readily and exactly achieved at low magnification.

In natural circumstances the larvae, having attached themselves to some warm-blooded animal, gorge from 1 to 3 days, then leaving the host they crawl into the soil and turn into immovable pupae. On the other hand insufficiently fed or unfed larvae are unable to develop further and remain in the larval stage for a fairly long time. We often observed that they survive in the larval stage over 30 days; in these cases they may again attack some warm-blooded animal. In general it may be said that larvae attaining to about 0.3 mm. (minus capitulum, measured alive) are already able to pass over the secondary stage.

As we have already noted in a previous report, the adults of the tsutsugamushi, captured in the endemic area, are not the same in color; some are yellowish red and others pale yellow or even grey. This has no relation to the sex or the size of the body. Though we were as yet unable to recognize their morphological differences, when unmounted, with exactitude, we surmised that the color might possibly be one of the points of specific differentiation in the adult stage, and assorted the adults according to their color in separate bottles to observe which species would issue from them. From the fact that the larva and artificially hatched nymph of *Tr. akamushi*

TABLE IV.

	Temperature.	Number of bottle.	Number of adults put into the bottle.	Date of collection.	Date of detection of larvae.	Number of larvae found.	Species of hatched larvae.
Yellowish red adults.........	Room temp.	I	14	8/26/18	9/18	1	*Tr. akamushi*
	22° C. (6/2 →)	II	54	5/28/19	7/7	2	"
	"	III	110	5/29/19	6/22	136	"
	30° C. (6/2 →)	IV	600	5/ 8/19	6/12	4	"
	"	"	"	"	6/13	6	"
	"	"	"	"	6/14	7	"
	"	"	"	"	6/16	2	"
	"	V	593	5/8–9/19	——	0	——
	"	VI	127	5/29/19	7/9	2	*Tr. akamushi*
	"	VII	201	5/30/19	6/17	143	"
	"	"	"	"	6/18	1	"
	"	"	"	"	6/19	75	"
	Room temp.	VIII	204	5/2 /19	7/8	2	"
	"	IX	760	5/4–5/19	——	0	——
	"	X	52	5/27/19	6/21	72	*Tr. akamushi*
	"	XI	191	5/30/19	7/7	ca. 200	"
	"	XII	41	5/31/19	7/8	ca. 100	"
Pale yellow adults.	22° C. (6/2 →)	I	45	5/4–5/19	——	0	——
	"	II	"	5/8–9–10–14/19	——	0	——
	"	III	19	5/28/19	7/5	2	*Tr. pallida*
	30° C. (6/3 →)	IV	?	"	7/4	7	"
	"	"	"	"	——	0	——
	"	V	70	5/30/19	7/4	4	*Tr. pallida*
	Room temp.	VI	7	5/27/19	7/2	2	"
	"	VII	30	5/29/19	7/3	2	"
	"	VIII	7	5/31/19	"	4	"

are always deeper in color than those of *Tr. pallida*, etc., we presumed that the yellowish red adults might be the parent of the former, and in fact it was so proven by subsequent examinations. The following table gives data in one of these experiments (Table IV).

Nymphs.

In the breeding experiments we have obtained nymphs of four species: *Tr. akamushi, pallida, palpalis* and *scutellaris.* The nymph of *Tr. akamushi,* immediately after it has emerged from the larval skin, measures from the tip of the crista metopica to the posterior margin (exclusive of the layer of body hairs) about 0.28–0.38 mm., the abdominal part attaining 0.24–0.34 mm. in length and 0.2 mm. in width. While kept in bottles with adequate food they may double in size after the lapse of one month. As to the size, form of the body and legs, palpi and hairs we cannot detect any definite difference among four species. The nymph of *Tr. akamushi,* however, is more deeply colored than the other three, but much paler than its adult and, though they increase in color after a certain period, they never exceed their adult in redness. The crista metopica and areola sensilligera may be to a certain degree morphological points of differentiation. In all species there is one pair of sensory hairs on the areola sensilligera, measuring about 135 microns. The distal part of the sensory hair is provided with a number of slender branches in each species. But on the proximal part there are, in *Tr. akamushi,* only traces of minute processes, while those of *pallida* show distinct spines. In *Tr. palpalis* and *scutellaris* we can also recognize spines, but they are less prominent than those of *pallida.* On the other hand, the part of the scutum posterior to the areola is raised conically on both sides, which is more marked in *Tr. akamushi* and *palpalis* than in *pallida* and *scutellaris.*

Adults.

So far we have succeeded in our breeding experiments in obtaining the adults of *Tr. akamushi, pallida* and *palpalis.* Artificially bred adults of *Tr. akamushi* are velvety and orange red or cinnabar red in color. Their legs are also tinged with red. A pair of sensory hairs bear on their proximal part minute denticulation, the rest being provided with lateral branches. The rostriform processes of the scutum behind the areola are more prominent in this species than in those of the other two. The adults of *Tr. pallida* and *pal-*

TABLE V.

Date.			Number of voles captured.	Daily average temperature in ° C.	*Tr. akamushi.*	*Tr. pallida.*	*Tr. palpalis.*	*Tr. intermedia.*	*Tr. scutellaris.*	Japanese harvest mite.
1921	January	13	1	1.8	0	7	19	0	0	0
		14	1	1.9	0	0	46	0	0	0
		17	1	1.7	0	9	104	0	0	0
		19	1	2.0	0	11	60	0	0	0
1920	February	23	1	−4.1	0	0	3	0	0	0
		26	1	−1.7	0	14	51	0	0	0
		28	2	−0.1	0	22	186	0	0	7
		29	1	0	0	2	73	0	0	6
1919	March	24	15	8.7	0	48	40	0	0	0
		27	5	3.5	0	284	374	0	0	2
	April	5	12	6.6	0	236	539	0	0	10
		17	9	8.2	0	127	200	0	0	5
		18	4	8.6	0	74	135	0	0	7
		26	3	14.2	0	100	134	0	0	0
		29	4	10.9	0	66	196	0	0	1
	May	5	3	12.7	0	297	205	0	0	3
		10	3	17.9	0	4	36	0	0	0
		17	3	13.6	0	135	77	0	0	2
		19	6	14.9	0	307	18	0	0	0
		22	7	8.5	0	291	81	0	0	2
		26	6	12.1	0	188	86	0	0	0
		30	12	19.0	0	272	10	0	0	0
		31	5	16.2	0	467	9	0	0	0
	June	15	4	13.4	0	132	0	0	0	0
		16	1	16.0	0	53	1	0	0	0
		18	2	18.8	0	144	3	0	0	0
		19	5	20.2	1	376	0	0	0	0
		20	6	21.0	17	308	3	0	0	0
		21	14	21.7	43	467	2	0	0	0
		22	10	21.1	39	139	0	0	0	0
		23	4	17.6	9	15	0	0	0	0
		24	10	19.6	48	108	0	0	0	0
		25	11	22.8	61	79	0	0	0	0
		26	8	16.8	34	97	0	0	0	0
		28	2	21.1	55	59	0	0	0	0
		29	9	21.7	48	12	0	0	0	0
	July	27	8	23.6	118	110	0	0	0	0
		28	12	22.9	81	7	0	0	0	0
		29	6	24.2	43	11	0	0	0	0
		31	3	26.0	0	5	0	0	0	0
	August	1	12	25.0	232	27	0	0	0	0
		2	19	24.0	264	92	1	0	0	0
		3	3	25.0	7	22	0	0	0	0
		4	4	26.3	330	102	0	0	0	0
		5	6	25.3	99	46	0	0	0	0

Date.		Number of voles captured.	Daily average temperature in °C.	*Tr. akamushi.*	*Tr. pallida.*	*Tr. palpalis.*	*Tr. intermedia.*	*Tr. scutellaris.*	Japanese harvest mite.
August	6	4	23.0	62	137	0	0	0	0
	7	5	23.9	101	246	0	0	0	0
	8	2	23.4	206	82	0	0	0	0
	9	5	23.5	290	109	0	0	0	0
	10	6	24.5	314	54	0	0	0	0
	11	3	25.9	6	11	0	0	0	0
	12	4	25.4	73	62	0	0	0	0
	13	7	25.4	19	22	0	0	0	0
	14	4	21.2	0	0	0	0	0	0
	16	11	17.9	27	30	0	0	0	0
	18	8	20.6	43	114	1	0	0	0
	19	5	22.9	45	43	0	0	0	0
	20	9	22.9	269	174	1	0	0	0
	21	6	21.8	429	96	2	0	0	0
	22	5	22.1	142	107	1	0	0	0
	23	5	23 3	72	16	0	0	0	0
	24	1	25.1	1	0	0	0	0	0
	25	4	25.8	14	42	1	0	0	0
	26	5	24.7	10	73	6	0	0	0
	30	8	26.8	40	30	0	0	0	0
	31	6	26.3	3	22	0	0	0	0
September	8	6	20.2	95	518	16	0	0	0
	9	4	21.9	124	202	7	0	0	0
	14	4	22.3	13	37	45	0	0	0
	15	4	24.9	28	74	15	5	0	0
	18	4	24.2	32	65	13	2	0	0
	20	6	21.4	28	1125	41	1	0	0
	22	6	22.9	14	73	0	0	0	0
	23	5	19.6	10	182	5	0	0	0
	24	4	16.8	15	209	4	0	0	0
	25	5	15.4	21	111	9	0	0	0
	26	6	17.8	0	18	1	0	0	0
	27	13	17.7	4	410	34	16	0	0
	28	5	17.1	1	99	109	0	0	0
	29	1	16.1	10	69	8	35	0	0
	30	6	16.0	5	222	149	0	0	0
October	1	4	18.2	6	115	31	116	0	0
	2	5	18.6	7	49	22	109	0	0
	3	6	17.4	8	263	55	525	0	0
	4	5	15.4	3	68	13	356	0	0
	5	4	14.4	2	27	5	13	0	0
	6	5	14.5	0	50	57	0	0	0
	10	3	16.0	0	2	0	0	0	0
	11	6	14.7	0	406	94	69	0	0
	12	11	15.9	1	487	37	8	0	0
	13	10	16.0	2	1222	126	198	0	2
	14	9	15.8	7	852	113	89	0	0
	15	6	15.3	0	110	23	20	0	0
	16	4	13.6	0	398	94	130	0	1
	17	5	13.3	0	25	9	46	0	0
	18	6	11.6	0	121	24	11	0	1

Date.			Number of voles captured.	Daily average temperature in °C.	*Tr. akamushi.*	*Tr. pallida.*	*Tr. palpalis.*	*Tr. intermedia.*	*Tr. scutellaris.*	Japanese harvest mite.
1920	October	19	2	10.5	0	85	51	1006	0	88
		20	1	11.3	0	5	0	0	0	0
		21	1	12.0	0	174	38	68	0	15
		24	2	9.4	0	120	51	112	0	24
		25	2	9.0	0	47	107	59	0	6
		26	2	12.8	0	6	167	62	0	32
		28	2	14.3	0	60	68	20	0	229
		30	1	11.3	0	46	7	6	0	0
1920	November	1	1	11.3	0	3	2	1	0	0
		3	2	11.6	0	196	108	17	0	407
		4	1	14.3	0	21	39	8	0	83
		6	2	5.6	0	88	275	65	0	603
		7	1	8.2	0	33	113	39	0	286
		8	1	13.0	0	43	78	16	0	25
		9	1	9.8	0	8	9	2	0	4
		10	1	8.8	0	42	32	0	0	1
		12	1	7.2	0	9	4	1	0	5
		15	2	5.6	0	30	151	2	0	12
		16	3	5.0	0	14	86	0	0	1
		17	3	3.8	0	24	55	0	0	1
		19	3	6.1	0	90	265	8	0	20
		23	1	3.1	0	3	33	0	0	66
		24	2	6.6	0	17	82	0	0	0
		25	2	9.6	0	1	131	0	0	0
		26	5	9.0	0	3	15	0	0	0
		27	1	7.8	0	22	410	0	0	1
		28	1	5.6	0	11	114	0	0	11
1920	December	4	2	5.2	0	70	105	0	0	1
		12	1	0.9	0	0	13	0	0	0
		22	1	2.9	0	20	68	0	0	0
		23	1	0.7	0	6	12	0	0	0
		24	1	1.5	0	5	15	0	0	0

palis are paler in color than that of *akamushi,* and their legs appear almost colorless. The proximal part of the sensory hairs bears minute spines. Besides these there are almost no specific differences in morphological respects with regard to their size, form, mouth apparatus, hairs, genital and anal opening, and epimeres, etc.

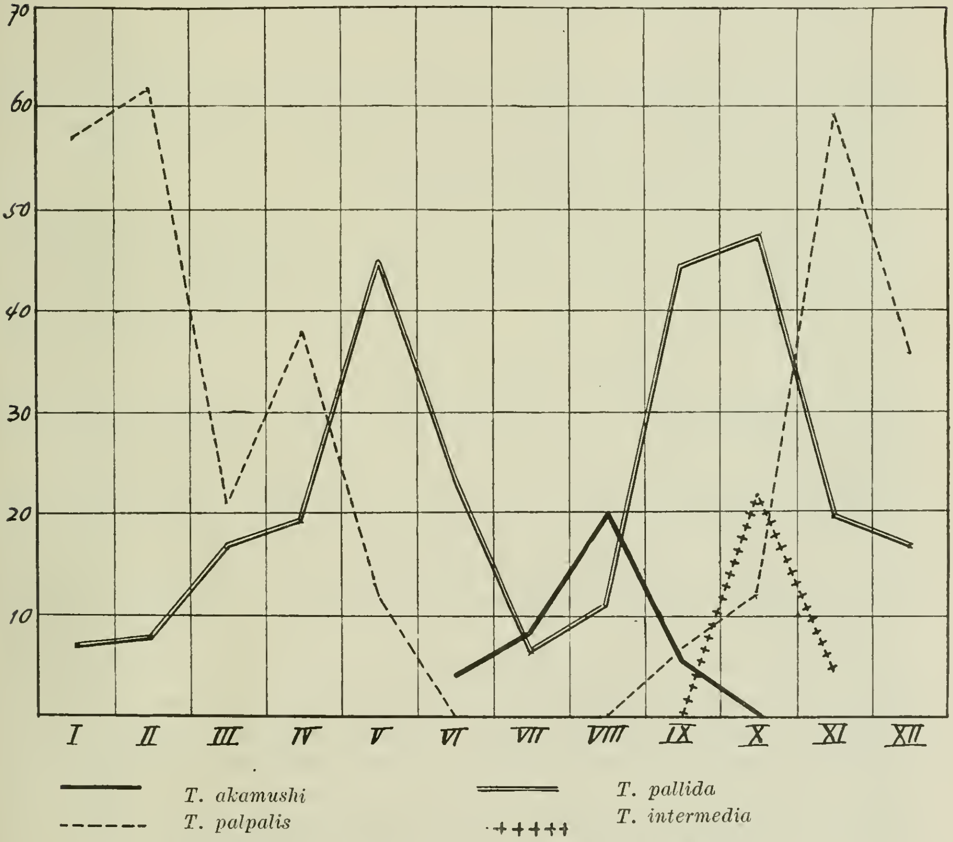
Nymphophane and teleiophane.

On primary and secondary immovable pupae there is on the dorsum anteriorly one conical chitinous process, which, standing out of the apoderma, pierces the original skin. It is not specific in tsutsugamushi, but is also found in the nymphophane and teleiophane of mites, which are called Akidani (Japanese harvest mite).

SEASONAL PREVALENCE OF EACH SPECIES OF TSUTSUGAMUSHI.

For the purpose of obtaining exact knowledge as to the seasonal fluctuations in the occurrence of each species of tsutsugamushi, we collected specimens almost throughout the year from the ear-lobes of voles, which are the principal hosts in endemic areas. We examined the mites in Yachi, Nukanome and Anato, all in Yamagata Prefecture. But for convenience sake we preferred Okiage in Yachi, the most infected and ill-reputed place for the disease in Yamagata Prefecture, in which all species are to be met with except *Tr. intermedia*. The daily catches were as follows (Table V):

The above numbers of each species of mites may be represented in the following curve:



Monthly fluctuations of each species of tsutsugamushi in Yachi, Yamagata Prefecture.

As is obvious from the above chart it is very interesting to find that each of the tsutsugamushi species have their own seasonal period of occurrence. *Tr. akamushi* was found in 1919 on the 19th of June for the first time. It soon increases in number, exceeding in July and August other species. In September, however, it decreases gradually, dwindling towards the middle of October, then disappearing entirely. *Tr. pallida,* on the other hand, is found at any time of the year, yet showing monthly fluctuations. It appears most abundantly in May and October. *Tr. palpalis,* in fair contrast to *Tr. akamushi,* disappears during the midsummer, but is found in large numbers in the cold seasons. *Tr. scutellaris,* however, makes its appearance only for a short period, from the last half of September to the end of November, when it again disappears entirely.

With regard to *Tr. intermedia,* which hitherto was never found in Yachi, we have no exact knowledge regarding its seasonal behavior. Nevertheless, from the fact that it was found abundantly early in September and also late in November in Arato, 20 miles south from Yachi, we may perhaps presume that it appears throughout the year, as is the case with *Tr. pallida.*

The fact that the larvae of each species of tsutsugamushi have their own period of appearance is of great significance from the medical point of view as to the epidemiology and endemiology of the human tsutsugamushi disease. Though Tanaka, in his precise observations, first concluded that the tsutsugamushi must be of two species, it was generally accepted that they occur throughout the year, a contradiction to the fact that people are stung by the mite only in the hottest part of the year. Now that we know that only one species, *Tr. akamushi,* coincides in its occurrence with that of the human tsutsugamushi disease, we may say with great certainty that only one species must be the actual transmitter of the germ to man and the others do not attack human beings habitually, hence they are innocuous to man, whether they do or do not carry the virus in themselves.

Besides the five species of tsutsugamushi still another one has been found on voles differing in many respects from the others. This has been already described by several investigators and especially in detail by Okumura (12) under the name Japanese Akidani (harvest mite). It is a deep red minute mite, measuring about the same in size as the tsutsugamushi. The chief morphological differences are to be seen in the dorsal shield and hairs of palpus and legs.

The posterior margin of the scutum is curved rearward like that of the European *Leptus autumnalis,* and the character and length of the hairs upon it differ remarkably from that of the tsutsugamushi. The hairs on articles I, II and III of the palpus are all provided with lateral branches. On leg III there are four long plain hairs (sensory hairs), one on the protarsus and the remainder on the tarsus, directed externally. This species of mite is met with on the ear-lobes of voles together with the tsutsugamushi in cold seasons as is seen in the above table. Our breeding experiments have brought its nymph and imago to light, both of which are more oblong in form than those of tsutsugamushi, but equally show distinct constriction between the cephalothoracic region and the abdomen. The body hairs are short, and pointed at the end. The areola is triangular and the sensory hairs show distinct spinal processes on their proximal parts.

In our previous report (13) we stated that the larva of *Trombidium holosericeum* is quite different from *Leptus autumnalis* Shaw, and that the parents of the latter must be of another species, contrary to the statement of Mégnin. From our observations we concluded that the larva of the Japanese Akidani, which very much resembles *Leptus autumnalis,* issued from adults as noted above. We may therefore suggest with great certainty that the parents of the latter might be kindred ones.

Hosts of tsutsugamushi.

As above mentioned it was reported at first by Tanaka, that only one species of tsutsugamushi mites attacks man. We have also collected about 140 specimens of the mites, which were found attached to human bodies. Thorough examination of these specimens revealed the fact that they were all *Tr. akamushi,* some of which caused the tsutsugamushi disease in the bitten individuals.

Moreover it is worthy of special mention, that when we were in Arato collecting the mites in the early part of September, 1919, none of our party was attacked by any mite, though we had not taken any protective measures, but rats, on the contrary, when brought into the same place, each bore hundreds of larvae of *pallida, palpalis* and *intermedia* on their ear-lobes within several hours. In fact in this district, though at an early date it had acquired a most evil reputation, no case of infection has been found for a long time, and the mites met with are of three species, excepting *Tr. akamushi* and *scutellaris.*

From this we may conclude that the only species which habitually attacks man must be *Tr. akamushi,* and those regions, in which *Tr. akamushi* is not found, may be defined as innocuous no matter how numerous the mites of other species may be. Though we cannot deny the fact that other species may sometimes attack man, as is asserted by some investigators, such cases must be very rare.

Most of the laboratory animals such as the monkey, rabbit, guinea pig, rat, field mouse, etc., are liable to be attacked by all species of tsutsugamushi. It is also reported by Hayashi (14), Hatori (15) and Kawamura (16) that fowls as well as other birds are very often bitten by various species of the tsutsugamushi mite.

TSUTSUGAMUSHI AND THE CAUSATIVE AGENT OF THE TSUTSUGAMUSHI DISEASE.

From the fact that *Tr. akamushi* may infect man by their bite, there is no doubt that this species of mite harbours the virus of the tsutsugamushi disease in itself and transmits it when it has occasion to feed. It has been proven by animal experiments with *pallida* and *intermedia* that they may also carry the virus in themselves. But as they do not bite man it may be said that there is no direct relationship between them and the occurrence of human tsutsugamushi disease.

The problem whether the adults harbour the virus in the pathogenic state in themselves or not has often hitherto been discussed by some investigators. However we were able to cause the disease in three fuscatus-monkeys out of eight after injecting subcutaneously an emulsion of about 1210 adults, together with deep colored and paler ones. It was recognized as tsutsugamushi disease not only from its clinical features and subsequent inoculation of the animals' blood into another monkey, but also immunologically by way of cross experiment with the virus taken from a typical case of the disease (17).

As the larva of tsutsugamushi does not usually suck on two animals successively during its life it undoubtedly acquires the virus by ovarian infection from its parents. It is so far evident, that the adult and the nymph are not noxious to man, as is the case with the larva, not because they do not carry the specific germ, but because they do not attack warm-blooded animals.

TR. AKAMUSHI AND THE OCCURRENCE OF THE TSUTSUGAMUSHI DISEASE.

Now the period of appearance of *Tr. akamushi* and its habit of attacking man being settled, the question with regard to the seasonal occurrence of the tsutsugamushi disease has been solved, and the tsutsugamushi disease may be defined as an endemic and seasonal disease in the strict sense of the word; it occurs only in the locality and the season at which the larva of *Tr. akamushi* makes its appearance. According to our observations this species of mite passes the winter mostly in the adult stage, and late in May of the next year these mature animals deposit their eggs in the soil, as they are found very often bearing eggs at that time. In June when the daily temperature reaches a certain degree, that is about 20° C. as the daily mean temperature, the hatching of larvae begins to take place. This is the case in Yamagata Prefecture about the 20th of June and thereafter. The oviposition may last till September, as the adults are then found with eggs and unfed larvae, too, still in fairly large number.

The periodical flooding of the river is certainly of great significance as regards the prevalence of the disease. Nevertheless it has no direct relationship to the outbreak of the disease, as was once assumed by Baelz (Überschwemmungsfieber), though most of the endemic regions lie near rivers and are subject to inundations in the spring. According to our breeding experiments, they require especially in the nymphal period some complex organic debris such as decayed vegetable substances for their food, and just these matters are afforded them after the inundations, favoring the development of this kind of mite.

According to Hatori (15) the tsutsugamushi disease in Formosa is found already in March and new cases may be encountered till November. This is perhaps due to the hot climate of the locality, where the temperature in March corresponds to that of June in Yamagata Prefecture. Moreover it is worthy of notice that the endemic districts in Formosa are not restricted to river sides, but extend to fertile plains and woodlands, either at the foot of mountains or on elevations free from inundations (Hatori).

NOMENCLATURE.

The five species of tsutsugamushi above mentioned have each morphological properties which distinguish them from each other and never show any transition form among them, and with respect to

TABLE VI.

Specific determination of five forms of tsutsugamushi in their larval stage.

Points for differentiation.		Name of species.				
		Trombicula akamushi (Brumpt.)	*Trombicula pallida* nov. sp.	*Trombicula palpalis* nov. sp.	*Trombicula intermedia* nov. sp.	*Trombicula scutellaris* nov. sp.
Color.....	Color of trunk	Deep orange-red or vermillion-red	Orange-red	Orange-red	Orange-red	Orange-red
Scutum or dorsal shield	Form	Rectangular; posterior angles not rounded	Posterior angles slightly rounded	Posterior angles distinctly rounded, but not in such a degree as in *Leptus autumnalis*	Posterior angles distinctly rounded, but not in such a degree as in *Trombicula palpalis*	Posterior angles distinctly rounded
	Position of posterior lateral hairs	Laterally and posteriorly to sensory hairs (pseudo-stigmatal organ)	Anteriorly and laterally to sensory hairs	Anteriorly and laterally to sensory hairs	Anteriorly and laterally to sensory hairs	Laterally and at times somewhat anteriorly to sensory hairs
	Sensory hairs	Proximal part naked; ratio of proximal and distal parts about 2 : 3	Proximal part with minute spines; ratio of proximal and distal parts about 1 : 2	Proximal part naked; ratio of proximal and distal parts about 1 : 3	Proximal part with minute spines; ratio of proximal and distal parts about 1 : 2	Proximal part with traces of minute spines; ratio of proximal and distal parts about 3 : 5
Hairs of trunk	Number of hairs* (on an average)	On dorsal surface.........37 On ventral surface.........28 Total.........65	.... 60 .... 53 113	.... 45 .... 46 91	.... 38 .... 38 76	.... 51 .... 40 91
	Number of dorsal hairs in the first row	Commonly 10	Commonly 14	Commonly 13	Commonly 12	12–13

	Number of lateral branches on convex side of dorsal hairs (in each row)	Commonly 12–13	Commonly 10–11	Commonly 11–12	15 and 17 or 14 and 17	Commonly 10–11
	Number of lateral branches on concave side of dorsal hairs (in each row)	Commonly 6	Commonly 4	Commonly 5	4 and 5 or 3 and 5	Commonly 6
	Length of dorsal hairs in the first row (on an average)	48 μ	43.3 μ	51.4 μ	57.5 μ	51.2 μ
	Size and form of lateral branches of dorsal hairs	Very thin and short, pointed at the end	Conspicuous and thickest among all, rounded at the end	Of middle size, pointed at the end	Conspicuous and thick, but thinner than that of *T. pallida.*	Of middle size, pointed at the end
Palpus	Hair on ventral surface of third segment	No lateral branch	No lateral branch	4 or 5 lateral branches	No lateral branch	No lateral branch
Occurrence	Period of occurrence in Yamagata Prefecture	From June to October; most abundant in July and August	Throughout the year; most abundant in May and October	Practically absent in summer; most abundant between October and April	Found in Arato and Nukanome in Yamagata Prefecture in September and November	Found in Yachi from September to November
Host	Kinds of hosts	Warm-Blooded animals, inclusive of human beings	Warm-Blooded animals, but commonly not human beings	Warm-Blooded animals, but commonly not human beings	Warm-blooded animals, but commonly not human beings	Warm-blooded animals, but commonly not human beings

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* Exclusive of those on the scutum, coxae (epimeres) and between the coxae.

their characteristics in dorsal shields, hairs, palpi, etc., are absolutely of fixed forms in each species under any circumstance or any climate. Besides this, they differ also biologically from each other; they have their own period of occurrence and show certain differences with regard to their host (Table VI). Though not as distinctly as in the larval stage there are certain discrepancies among their nymphs and adults.

From these entomological points of view we propose to separate these forms of tsutsugamushi as different species, thus:

1. Trombicula akamushi (Brumpt) 1910.
2. Trombicula pallida Nagayo 1919.
3. Trombicula palpalis Nagayo 1919.
4. Trombicula intermedia Nagayo 1920.
5. Trombicula scutellaris Nagayo 1920.

For the present we adopt here the generic name *Trombicula* instead of *Leptotrombidium* which we proposed in a previous report in this paper, because Berlese's description of his Trombicula coincides in generic determination with that of our Leptotrombidium in many respects. Whether the latter is to be regarded as a synonym of the former or to be introduced as a subgenus, depends on further comparative studies of these kinds of mites.

SUMMARY.

1. According to our present knowledge there are at least five species among the mites which have been defined as tsutsugamushi. They differ morphologically as well as biologically from each other and never show any transition forms.

2. The specific differences in the morphology are most distinct in their larval stage; the color of the body, form and number of body hairs, the scutum and hairs upon it, and the arrangement of the latter, and character of the hair on the third article of the palpus, etc., being the main points of differentiation.

3. Practically only one of these species, *Tr. akamushi*, is of significance in the occurrence of human tsutsugamushi disease. From the seasonal point of view the hatching of larvae of the species from the eggs and the larval development coincides with the outbreak of the tsutsugamushi disease, in other words: the tsutsugamushi disease is observed only in the season in which the development of the larvae of *Tr. akamushi* takes place. Also the geographical distribution of the tsutsugamushi disease corresponds to that of *Tr. akamushi*, not to the distribution of other species.

4. The recognition of so-called endemic districts (noxious places) may be effected by the detection of *Tr. akamushi*, and not of others. With this entomological knowledge it is of great importance in the carrying out of prophylactic measures as well as from a financial point of view in agriculture to demarcate the dangerous places.

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EXPLANATION OF PLATES.

PLATE XXXIV.

FIG. 1. *Trombicula akamushi,* larva, dorsal aspect × 200.
FIG. 2. *Trombicula akamushi,* ventral aspect × 200.
FIG. 3. *Tr. pallida,* larva, dorsal aspect × 200.
FIG. 4. *Tr. pallida,* ventral aspect × 200.
FIG. 5. *Tr. palpalis,* larva, dorsal aspect × 200.
FIG. 6. *Tr. palpalis,* ventral aspect × 200.

PLATE XXXV.

FIG. 7. *Tr. intermedia,* larva, dorsal aspect × 200.
FIG. 8. *Tr. intermedia,* larva, ventral aspect × 200.
FIG. 9. *Tr. scutellaris,* larva, dorsal aspect × 200.
FIG. 10. *Tr. scutellaris,* larva, ventral aspect × 200.
FIG. 11. Palpus of larva of *Tr. akamushi,* ventral aspect × 950.
FIG. 12. Palpus of larva of *Tr. palpalis,* ventral aspect × 950.

PLATE XXXVI.

FIGS. 13–17. Dorsal hairs of larvae. Those in the first row (a) and on the posterior margin of the body (b) × 1150.

FIG. 13. *Tr. akamushi.*
FIG. 14. *Tr. pallida.*
FIG. 15. *Tr. palpalis.*
FIG. 16. *Tr. intermedia.*
FIG. 17. *Tr. scutellaris.*

PLATE XXXVII.

FIGS. 18–22. Scuta of larvae.
FIG. 18. *Tr. akamushi* × 770.
FIG. 19. *Tr. pallida* × 900.

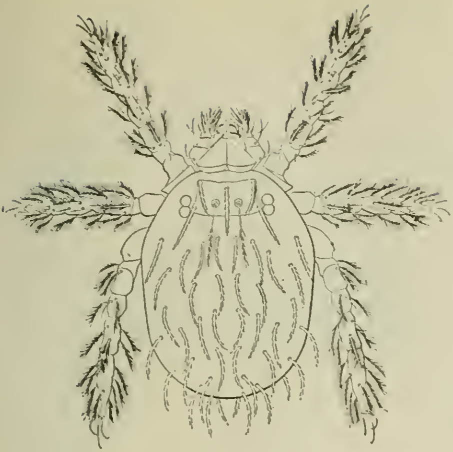


Fig.1

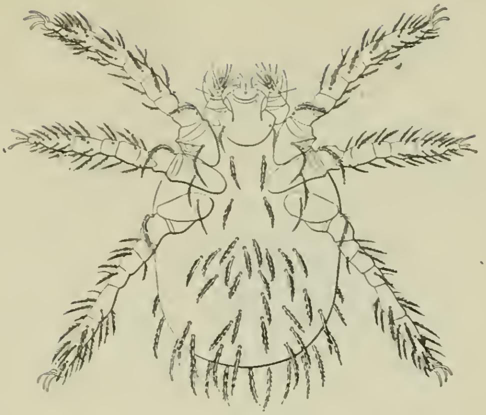


Fig. 2

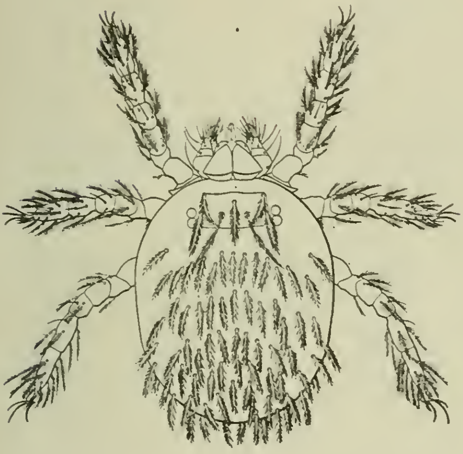


Fig. 3

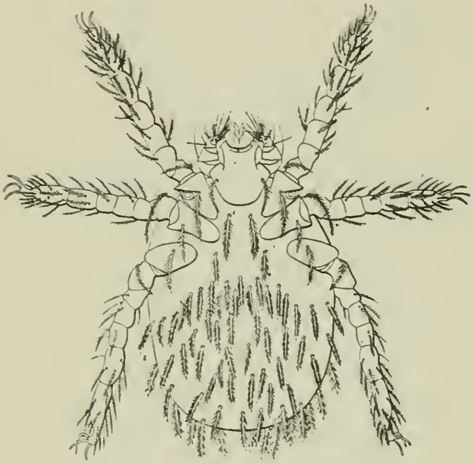


Fig. 4

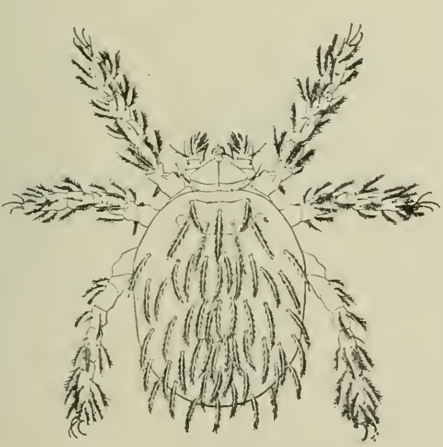


Fig. 5

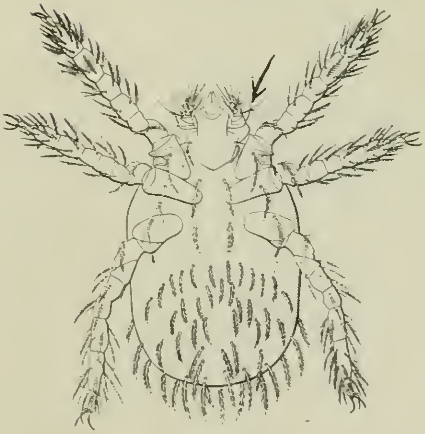


Fig. 6

PLATE XXXIV

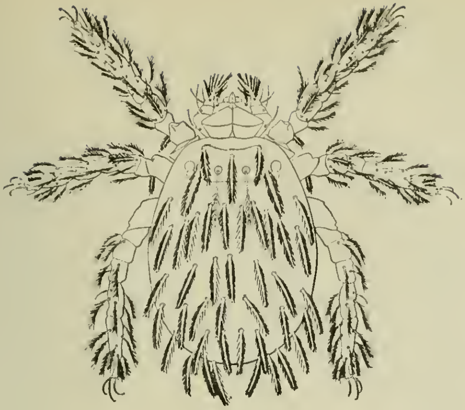


Fig. 7

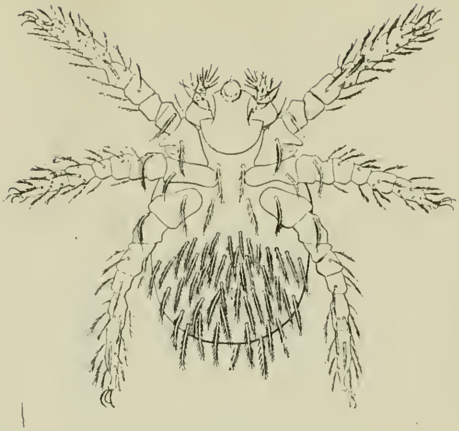


Fig. 8

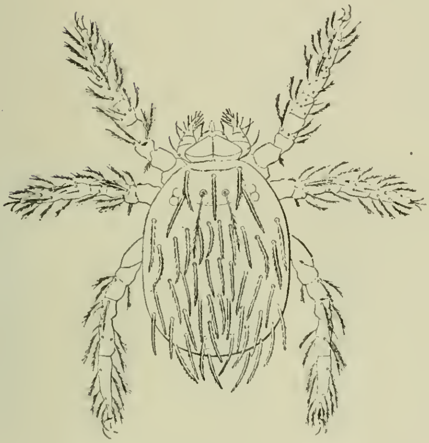


Fig. 9

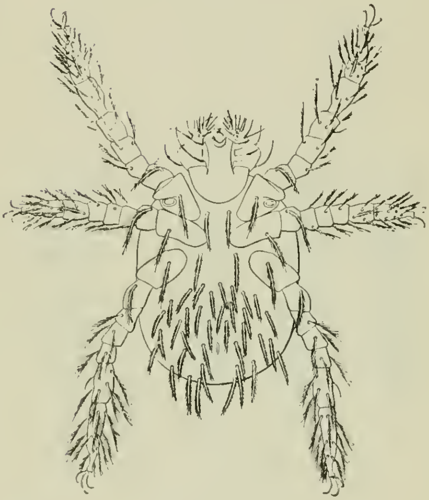
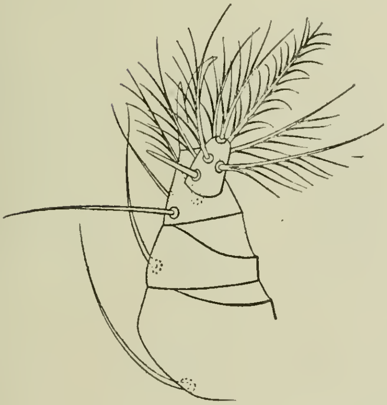


Fig. 10



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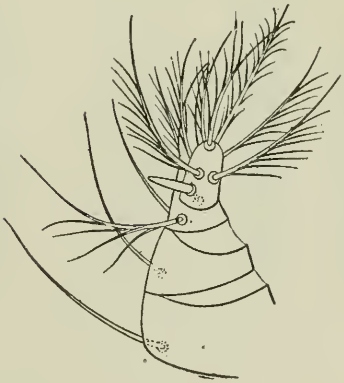
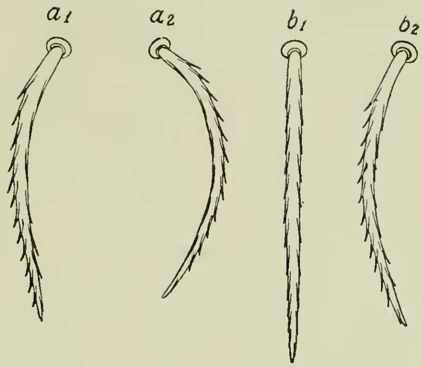


Fig. 12

PLATE XXXV



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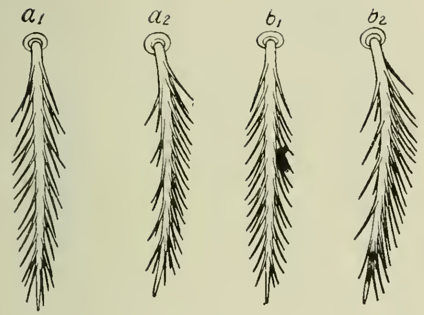

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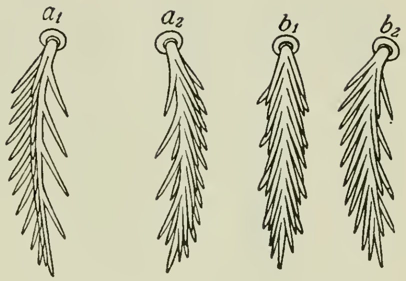

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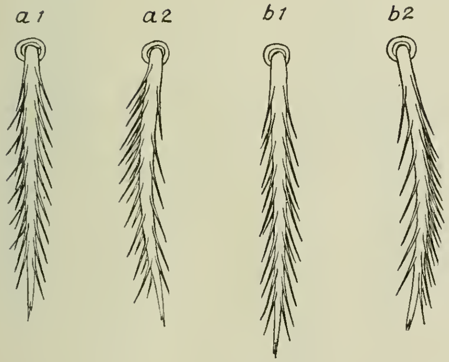

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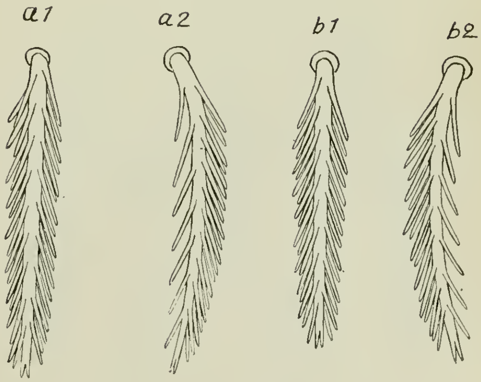
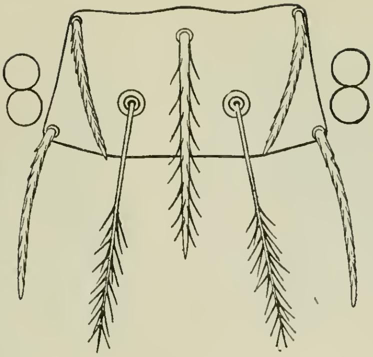


Fig.16

PLATE XXXVI



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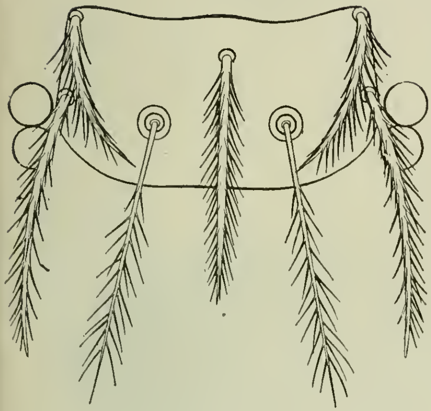
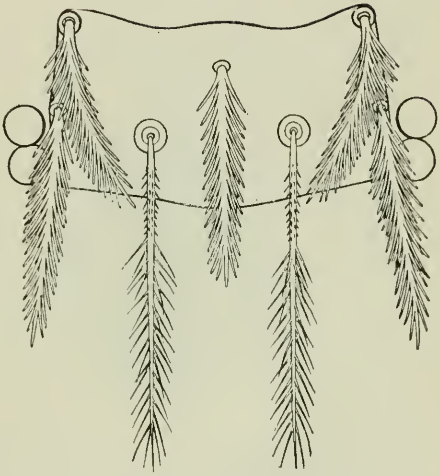


Fig.20



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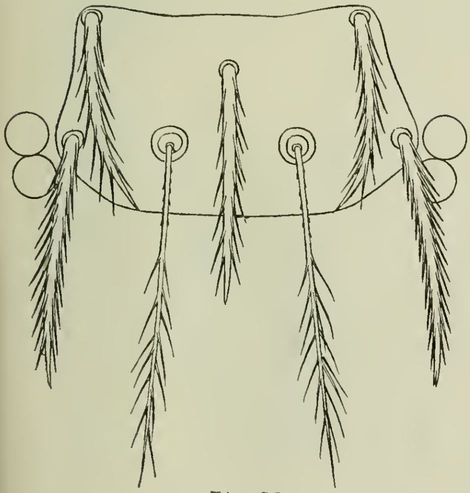

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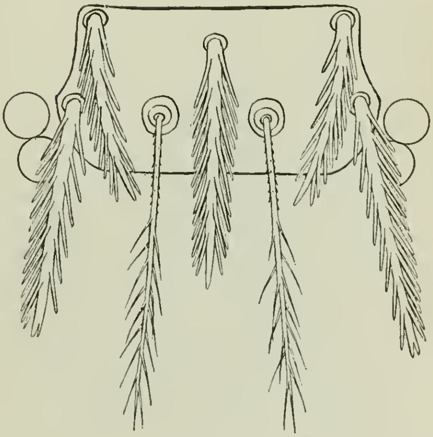
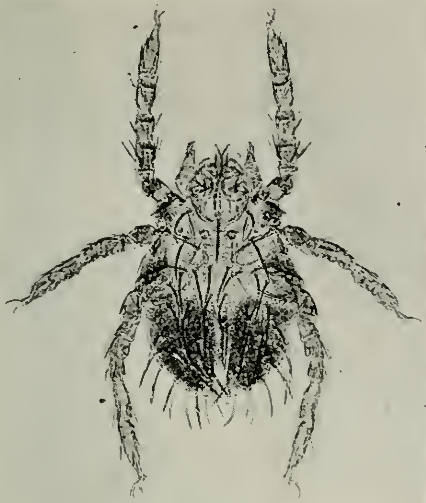


Fig.21

PLATE XXXVII



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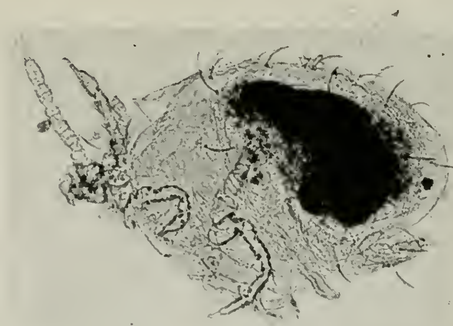

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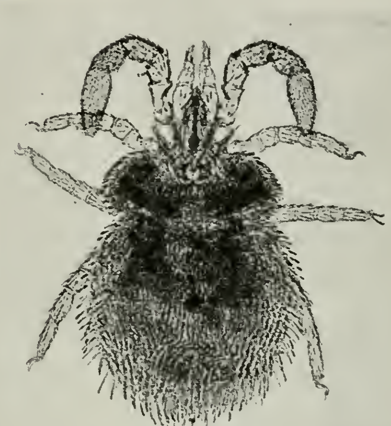

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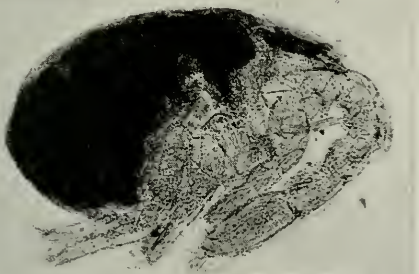

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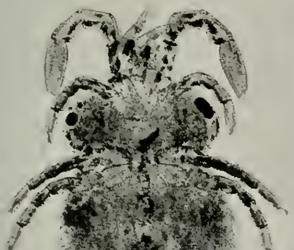

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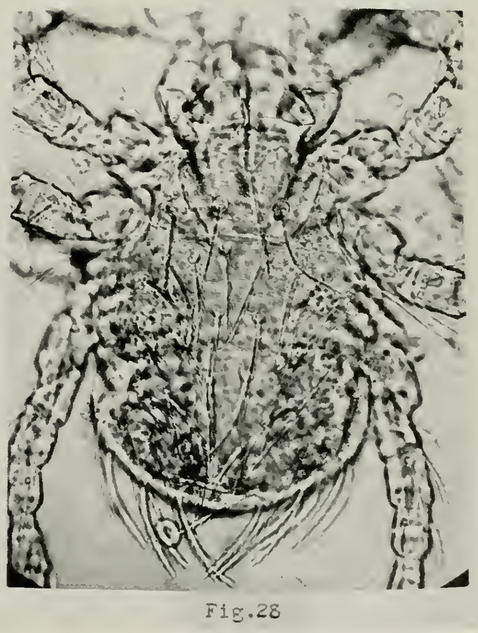
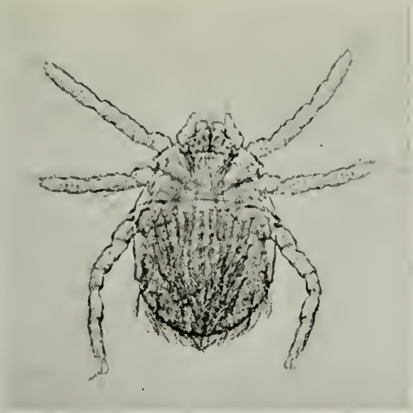


Fig.28

PLATE XXXVIII



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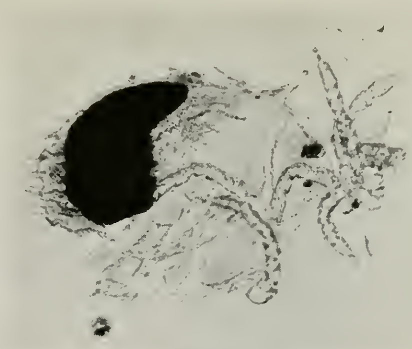

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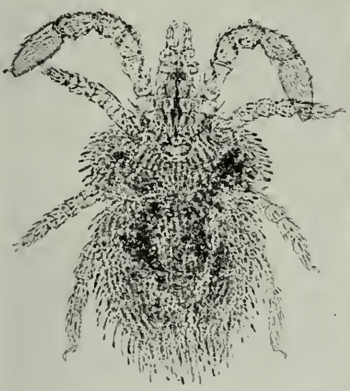

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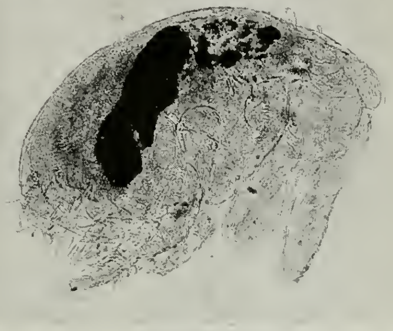

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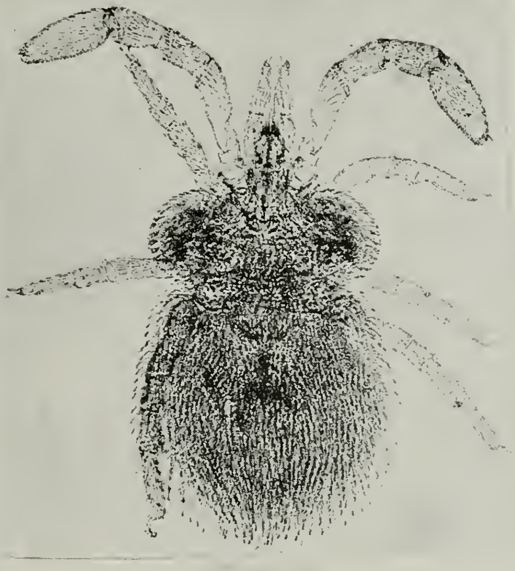
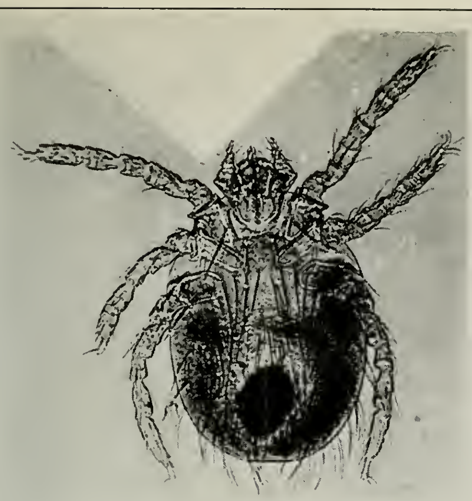


Fig.33

PLATE XXXIX



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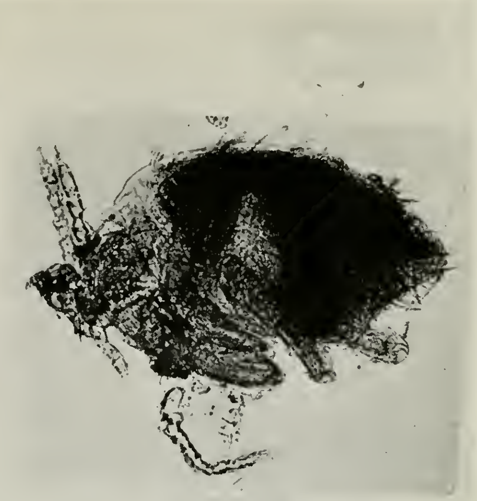

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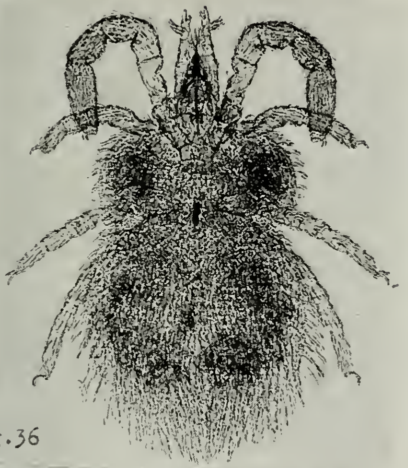

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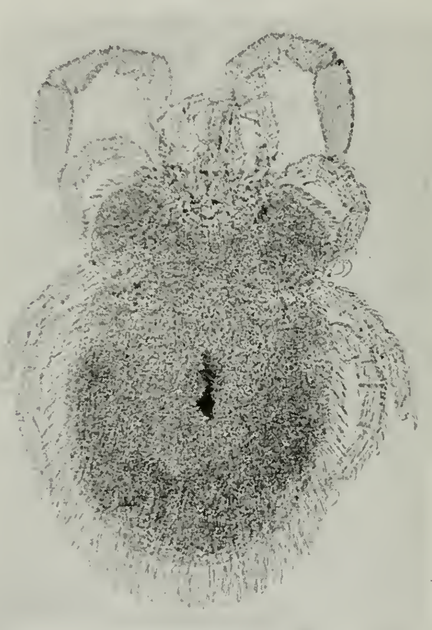

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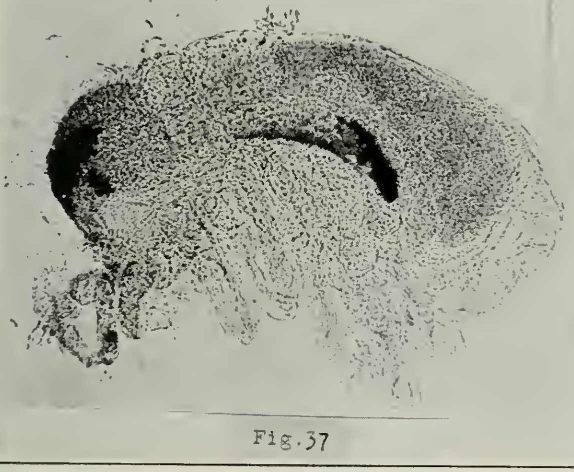
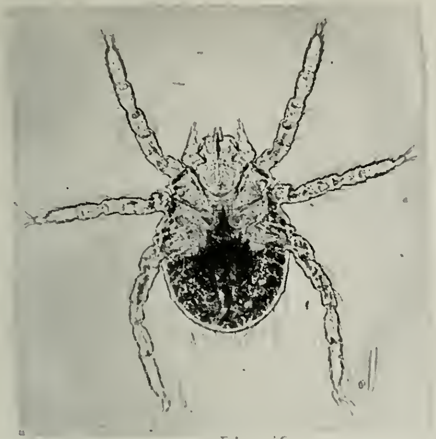


Fig.37

PLATE XL



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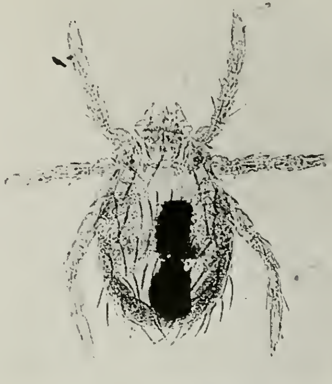
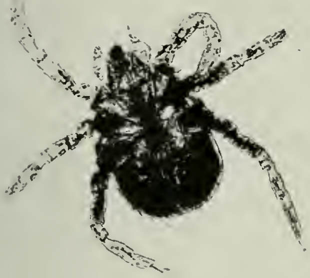


Fig.40



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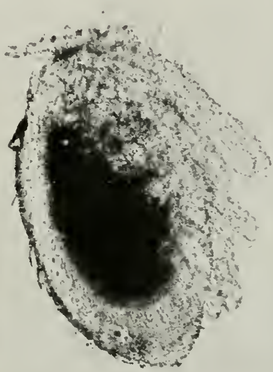

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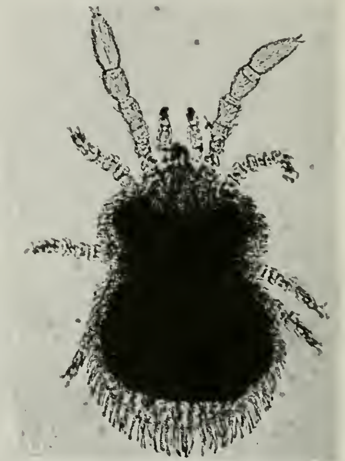

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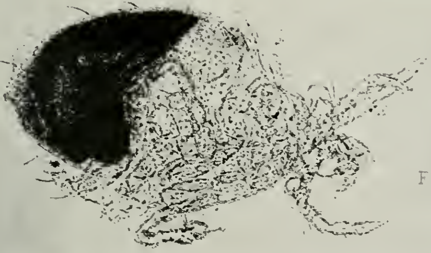

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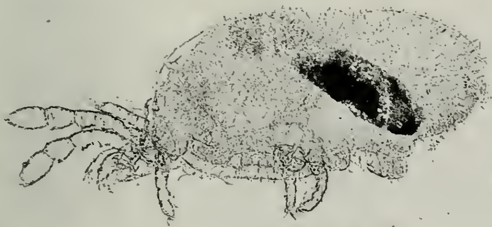

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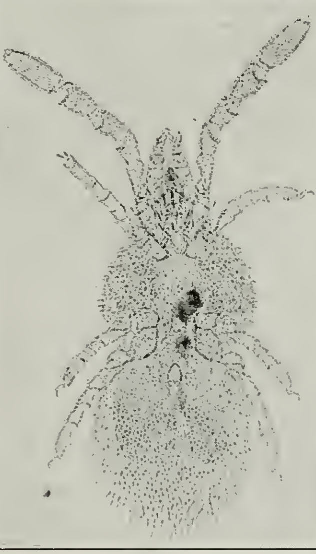

Fig.46

PLATE XLI

Fig. 20. *Tr. palpalis* × 830.
Fig. 21. *Tr. intermedia* × 830.
Fig. 22. *Tr. scutellaris* × 850.

Plate XXXVIII.

Life cycle of *Tr. akamushi* (artificially bred specimens).

Fig. 23. Larva × 200.
Fig. 24. Nymphophane × 180.
Fig. 25. Nymph × 100.
Fig. 26. Teleiophane × 116.
Fig. 27. Prosopon × 70.
Fig. 28. Larva × 420.

Plate XXXIX.

Life cycle of *Tr. pallida* (artificially bred specimens).

Fig. 29. Larva × 180.
Fig. 30. Nymphophane × 150.
Fig. 31. Nymph × 100.
Fig. 32. Teleiophane × 110.
Fig. 33. Prosopon × 100.

Plate XL.

Life cycle of *Tr. palpalis* (Figs. 35–38, artificially bred specimens).

Fig. 34. Larva (captured specimen) × 210.
Fig. 35. Nymphophane × 180.
Fig. 36. Nymph × 125.
Fig. 37. Teleiophane × 125.
Fig. 38. Prosopon × 125.

Plate XLI.

Fig. 39. Larva of *Tr. intermedia* (captured specimen) × 180.
Fig. 40. *Tr. scutellaris,* larva (captured specimen) × 180.
Fig. 41. *Tr. scutellaris,* nymphophane × 130.
Fig. 42. *Tr. scutellaris* nymph (artificially bred specimen) × 87.
Fig. 43. Japanese harvest mite larva (artificially bred specimen) × 180.
Fig. 44. Japanese harvest mite nymphophane × 160.
Fig. 45. Japanese harvest mite teleiophane × 100.
Fig. 46. Japanese harvest mite prosopon (captured specimen) × 100.

THE VITALITY OF THE PEOPLES OF AMERICA.*

By RAYMOND PEARL.

(Received for publication September 17th, 1921.)

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I.

The General Problem of Racial Amalgamation.

Science tries ever to peer into the future. Reasoning from the solid basis of human experience in general, that like antecedents are followed by like consequences, it is the constant endeavor of science so to formulate, classify and codify the relations which have in the past subsisted between antecedents and consequences as to be able to pick out the sets which show the maximum degree of constancy, with the thought that then the future may be predicted, if not with absolute certainty, at least with a high degree of probability. Nowhere is the desire to estimate what the future has in store stronger than in the case of matters relating directly to human actions, emotions and thoughts. We know that we are on our way. But where are we going?

This general interest of man in his future social, physical, and mental evolution crystallizes in a specific way for us here and now in the problem of the future of American civilization. That magnificent experiment in human evolution which is called the United States of America has, from its beginning, been at once the embodiment of the loftiest hope and the deep despair of different groups of people. In theory it has always offered what is perhaps the greatest boon intelligent human beings ever crave, equality of opportunity.

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* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, The Johns Hopkins University, No. 43.

This investigation was made, and the paper prepared at the request and with the financial aid of the Society of American Peoples of New York.

The ideal of freedom of opportunity has probably been as consistently maintained in fact, as would in any case be possible, human nature being what it is. Our gates have been open, with some slight and on the whole insignificant exceptions, to all and sundry. Good, bad and indifferent people have been free to settle here, and have made the fullest use of the freedom. Meantime the country has prospered mightily in material ways, and not insignificantly in things of the spirit, such cheerful pessimists as Mr. Mencken to the contrary notwithstanding. What vexes the mind is the consideration of the question as to whether the abounding blessings which on the whole have associated themselves with our country have appeared because of, or in spite of, the nearly complete abandon with which all kinds of people have been permitted, indeed encouraged, to join in the enterprise. According to one's views as to the answer to this question he is either fairly satisfied or somewhat disturbed as he looks towards the future.

The essential problem with which the present discussion has to do is that of the racial composition, and biostatistical characteristics in general, of the probable future population of this country. Our judgment on this problem must be determined by a careful examination of what has happened in the past together with what is now going on.

Our problem is purposely stated in this way to emphasize the fact that basically the problem of the future of America is simply one restricted phase of the general problem of population. Whether racial amalgamation or fusion occurs or not, gross population growth goes on steadily. Strange, and indeed inconceivable, as it may appear to many persons, the United States is going to be sometime a densely crowded country, where each individual, consciously or unconsciously, will be challenging the right of every other individual to existence. And, by every present indication, the time when this pressure of population will be definitely felt in this country is not so far away. Indeed in terms of the sort of time base by which evolutionary processes are commonly measured, the time of our overcrowding is right upon us. In less than two centuries we may expect a degree of pressure of population upon means of subsistence which will make the everyday life of everybody a different thing entirely from the prodigally wasteful business it now is.

The basis of this time prediction is found in certain mathematical studies which have been in progress in the writer's laboratory (1) during the past two years. Only a small part of our results have yet been published, but I shall here anticipate some of the as yet unpublished material. Pearl and Reed pointed out that the following conditions should be fulfilled by any equation which is to describe adequately the growth of population in an area of fixed limits.

1. Asymptotic to a line $y = k$ when $x = +\infty$.
2. Asymptotic to a line $y = 0$ when $x = -\infty$.
3. A point of inflection at some point $x = \alpha$ and $y = \beta$.
4. Concave upward to the left of $x = \alpha$ and concave downward to the right of $x = \alpha$.
5. No horizontal slope except at $x = \pm\infty$.
6. Values of y varying continuously from 0 to k as x varies from $-\infty$ to $+\infty$.

In these expressions y denotes population, and x denotes time.

An equation (2) which fulfills these requirements is

$$y = \frac{be^{ax}}{1 + ce^{ax}}, \tag{i}$$

when a, b and c have positive values.

In this equation the following relations hold:

$$x = +\infty, \qquad y = b/c, \tag{ii}$$
$$x = -\infty, \qquad y = 0. \tag{iii}$$

Relations (ii) and (iii) define the asymptotes. The point of inflection is given by $1 - ce^{ax} = 0$, or

$$x = -1/a \log c, \qquad y = b/2c. \tag{iv}$$

The slope at the point of inflection is $ab/4c$.

The general characteristics of the curve given by equation (i) are shown graphically in Fig. 1.

It was recognized that equation (i) gives but a first approximation to one special case of a general law of population growth. We have since developed the mathematical theory much further, and have arrived at a completely general equation, which accounts adequately for cyclical periodicity in population growth, asymmetry of point of

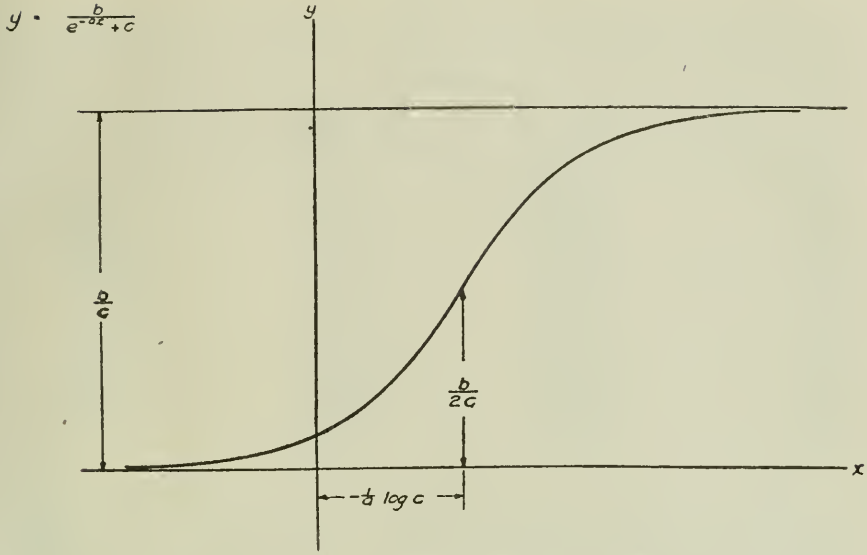

FIG. 1. General form of population growth curves given by equation (i).

inflection, periods of declining population, etc. This will be published shortly, it is hoped.

The more intensive researches have shown, however, that for countries showing normal growth within a single cultural epoch equation (i) is not greatly to be improved upon. The closeness with which it describes the known facts for the United States is shown in Table I, and graphically in Fig. 2.

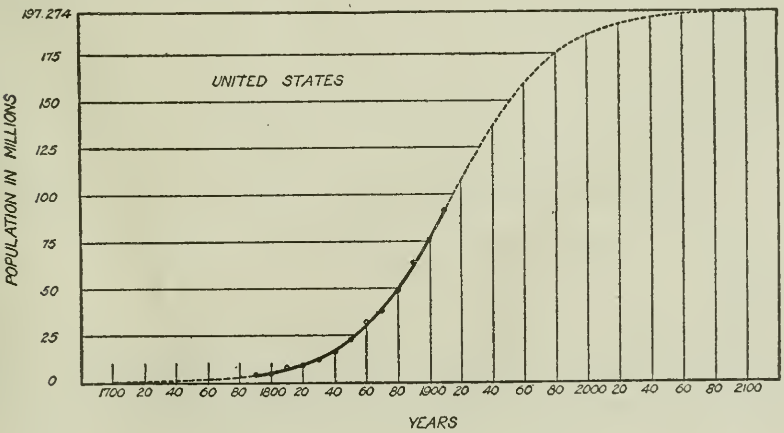

FIG. 2. The growth of the population of the United States.

TABLE I.

Results of fitting population data of the United States, 1790 to 1910, by equation (I).

Year.	Observed population.	Calculated population by equation.	Error.
1790.........	3,929,000	3,929,000	0
1800.........	5,308,000	5,336,000	+ 28,000
1810.........	7,240,000	7,228,000	− 12,000
1820.........	9,638,000	9,757,000	+ 119,000
1830.........	12,866,000	13,109,000	+ 243,000
1840.........	17,069,000	17,506,000	+ 437,000
1850.........	23,192,000	23,192,000	0
1860.........	31,443,000	30,412,000	−1,031,000
1870.........	38,558,000	39,372,000	+ 814,000
1880.........	50,156,000	50,177,000	+ 21,000
1890.........	62,948,000	62,769,000	− 179,000
1900.........	75,995,000	76,870,000	+ 875,000
1910.........	91,972,000	91,972,000	0

The heavy portion of the curve covers the region of known facts given by the census counts indicated on the diagram as small black circles. The light dotted portion of the curve indicates the probable future population growth.

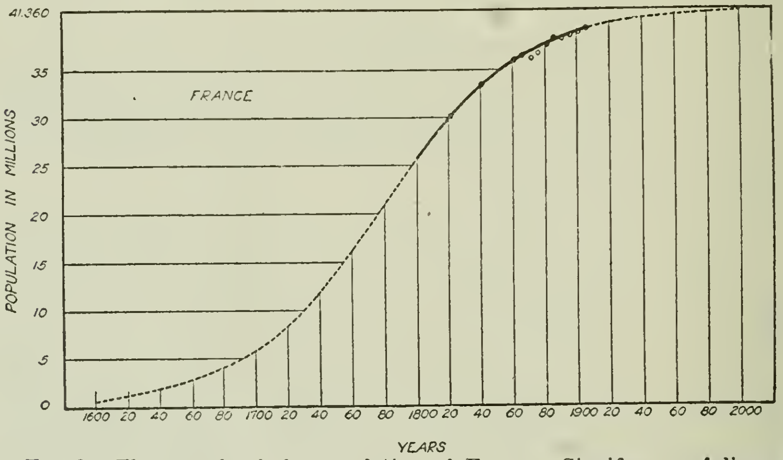

FIG. 3. The growth of the population of France. Significance of lines as in Fig. 2.

The closeness with which the curve fits the known facts, coupled with the fact that it substantially fulfils the *a priori* necessary conditions for a true organic law of population growth, gives confidence in its power to predict future events. In unpublished work we have shown that curves essentially like equation (i) describe with virtually perfect precision the known facts of the population growth for practically all civilized countries for which any census data exists. As further examples Figs. 3 and 4 are inserted which show that the data on population growth in France and in Serbia are described by this curve just as well as are those of the United States.

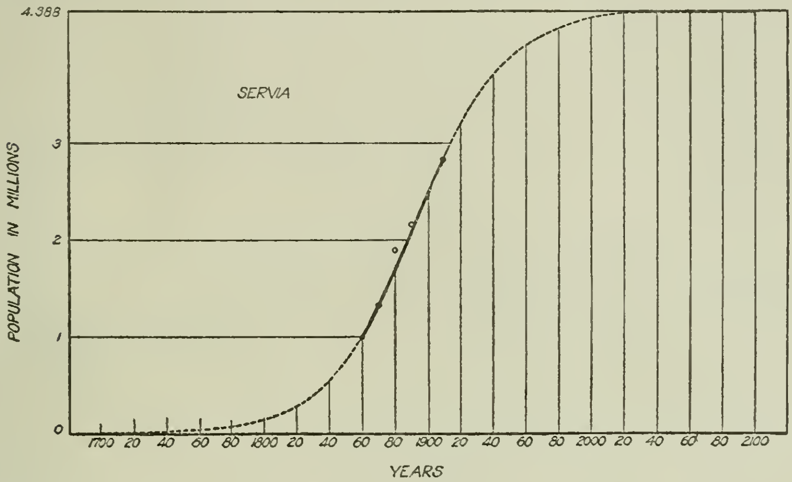

FIG. 4. The growth of the population of Serbia.

Returning to the consideration of the United States we see that, so far as we may rely upon present numerical values, the United States has already passed its period of most rapid population growth, unless there comes into play some factor not now known and which has never operated during the past history of the country to make the rate of growth more rapid. This latter contingency is improbable. The population at the point of inflection works out to have been 98,637,000, which was in fact about the population of the country in 1914.

The upper asymptote given by (i) has the value 197,274,000 roughly. This means that according to equation (i) the maximum population which continental United States, as now areally limited, will have will be roughly twice the present population. This will seem to some a small estimate. It is so easy, and most writers on population have been so prone, to extrapolate population by a geometric series, or by a parabola or some such purely empirical curve, and arrive at stupendous figures, that calm consideration of real probabilities is most difficult to obtain.

Now 197,000,000 people will require, on the basis of our present food habits, about 260,000,000,000,000 calories per annum. The United States during the seven years 1911–1918 produced as an annual average, in the form of human food, *both primary and secondary* (i.e., broadly vegetable and animal), only 137,163,606,000,000 calories per year (3). So that unless our food habits radically change and a man is able to do with less than 3000 to 3500 calories per day, or unless our agricultural production radically increases, it will be necessary when our modest figure for the asymptotic population is reached, to import nearly or quite one half of the calories needed by that population. It seems improbable that the population will go on increasing at any very rapid rate after such a condition is reached.

East (4) has lately made a critical examination of the agricultural limitations to the population of the United States, and concludes "that the agricultural possibilities in this country are not so great as they are generally supposed to be, and . . . even if these possibilities were fulfilled wholly the population the country could support is not enormously greater than the figures Pearl and Reed have set."

Fig. 2 also shows that we shall be uncomfortably close to the asymptotic population by 2100, a time at which the great-grandchildren of a number of persons now living will be components of the population.

What *kind* of a population will that be? What elements now in the population will then, and in the succeeding years, be the dominant ones? Or, will none of the racial groups now so distinct in our population exist as such then, having in the meantime fused and amalgamated, one with the other, till there remains only a biologically homogeneous whole? No one can answer these questions positively to-day. They are of the future. But we can examine present *tendencies* and past experience. It is to that task that this discussion is addressed. Many others have, of course, written on these problems. Notable papers were those of Mayo-Smith (5). Others who have written along similar lines are Michaud and Giddings (19), Giddings (20), Fishberg (21), Ripley (22), and Alleman (23). But since no recent writer has, so far as I am aware, approached the problem from at all the same point of view as that of the present study, no attempt will be made to review the literature systematically. The point of view throughout this paper is the formulation of the essential problems on which investigation should be, and profitably could be made, rather than any attempt to solve problems. In such formulations there will, of necessity, be clearly pointed out the appalling gaps in our official statistics. Many, indeed most, of the problems to be discussed can only be solved when there is a really adequate system of national bookkeeping, in respect of human life. In pointing out *lacunae* in our national biostatistics the animating motive will be wholly constructive and hopeful of future improvement, and not at all the contrary in respect of either persons or institutions. In general no one is more desirous than the officials charged with the collection and compilation of our national statistics that these statistics should be adequate, in the fullest sense of the word.

II.

Criteria of Biological Americanization.

In a strict sense there are but three general criteria by which the purely biological attributes of a population may be estimated or measured. These are:

1. Somatic physical.
2. Somatic psychological.
3. Biostatistical.

Logically, in a broad sense, all of these may be subsumed under the general title anthropological, but nothing particular is gained by so doing.

In the first category, somatic physical, will be included all those physical attributes of the individuals composing the population, which characterize them as animals, such as hair color, eye color, cephalic index, rate of growth, and so on, right away through the whole gamut of topics commonly designated physical anthropology.

In the second category will be included such attributes as intelligence, habits, feelings, emotions, and such other psychological matters as are capable of definition and measurement with greater or less precision, and at the same time exhibit racial variations of some degree of constancy and specificity.

With neither of these first two criteria shall we deal here, because they have been fully treated by specialists in these particular fields.

The biostatistical criteria form the subject matter of the present discussion. It will be well to examine their scope with some particularity before entering upon detailed treatment of the data.

From the point of view of the population problem, there are two biological phenomena of fundamental importance, *reproduction* and *death*. To study changes in population, of any sort whatever, we need comprehensive and accurate data about each separate element of both of these phenomena. To study progressive or retrogressive secular changes in population the most useful datum we can have is one which combines within itself the essential variations of both reproduction and death. Such a figure I have called (6) the " vital index " of a population.

We have then the following scheme of essential biostatistical criteria of changes in a people.

STATISTICS OF

- *A. Reproduction.*
  - 1. Marriages.
  - 2. Divorces.
  - 3. Births.
    - *a.* Living.
    - *b.* Still.
    - *c.* Illegitimate.
- *B. Death.*
  - 4. Deaths.
- *C. Vital Index.*
  - 5. Birth/Death ratio.

Each of the five groups of this scheme have, of course, many subsidiary elements, some of which we shall be able to consider.

There is one general defect of American vital statistics which so constantly will make itself felt in all the discussion which is to follow that it will be well to say what needs to be said about it, once and for all, at the start. The only index of race stock which we have anywhere in our statistics is such as may be inferred from facts as to the nation in which birth occurred. From the point of view of anything approaching exact science this is a most serious defect.

In the first place, in a number of places besides the United States, the population making up the nation includes a number of perfectly distinct racial stocks. Thus a Canadian, in our vital statistics, is just a Canadian, meaning that he was born in the Dominion of Canada, a political entity. In fact he may be an Indian, a Frenchman, an Englishman, an Eskimo, or of any one of a number of other ethnically distinct subdivisions of the human race.

In the second place, there is one highly important racial group, about which we are legally prevented from getting any biostatistical information. I refer to the Jews. Here, owing to the fact that what is by all odds the biologically purest of all civilized races happens to be also homogeneous in respect of religion, we apparently can have no statistics about it in this country. Whenever an attempt is made, as has repeatedly been the case, to get legislation which will permit the separate statistical enumeration of the Jews, it is blocked by the contention, on the part of some distinguished leaders of the race, that Jewry is a religion and nothing but a religion and does not connote a race. On the other hand others contend that the Jews constitute a highly homogeneous race. By all standards of common sense and anthropology the latter is of course the fact. But leaving quite aside all discussion of verbal definitions about race and religion, the plain fact cannot be successfully controverted that the Jews constitute a highly homogeneous group of people in the American commonwealth. It is for this reason and this alone, I take it, that every competent vital statistician agrees as to the great desirability of having statistical information about them. It is greatly to be hoped that in the not distant future this may be accomplished. So far as an entirely dispassionate and unprejudiced observer can see the Jews themselves could lose nothing, and would gain much if this reform could be established.

In the third place no attempt is made in our vital statistics to follow race stocks, even by nation of birth, for more than one generation. In the case of birth statistics we have the birthplace of the propositus and his parents. In the case of death statistics, we have the same information. In the latter case the information obviously extends further back in point of absolute time, but not in point of generations.

The vital statistician in this country has, perforce, to get into an attitude of mind in which he is grateful for what favors are accorded him. Serious as are the *lacunae* which have been mentioned, it is still possible to draw some reasonably accurate conclusions about race stocks in this country. In other words the case is not quite so bad as it might be. The following statement from "Birth Statistics for the Birth Registration Area of the United States, 1918" (p. 11), "Except for parents born in the United States, however, the country of birth undoubtedly does indicate usually the race stock," unquestionably represents a fact.

We may now turn to the more detailed consideration of the several biostatistical criteria of the changes of populations.

III.

Marriage and Divorce.

Under our existing conditions of law and custom marriage is a substantially essential prerequisite and concomitant of *normal* human reproduction. This does not mean, of course, that spermatozoa and ova will not successfully unite without benefit of clergy. Too often, alas, they do. But anyone who supposes that *normal* human reproduction goes on in our civilization outside of the marital state should examine the statistics of abortions, stillbirths, congenital malformations, and earliest infant mortality, in the cases respectively of legitimate and illegitimate unions. He will find the conditions in the case of illegitimate unions far indeed from biological normality. Consequently it is essential in the statistical discussion of reproduction to take such a factor into account.

There is furthermore in the present connection an especial reason for discussing marriage statistics. Vast quantities of ink have been spilled in the discussion of the " melting pot " in America. It would be unbelievable if it were not true, in view of the interest in this subject, and its obvious importance as a factor in national well-being, that no data are available by which the degree or extent of the " melting " or racial fusion can be measured on a national scale. What makes the case more curious is the fact that the initial element in the fusion is so easily and directly capable of measurement. The first step in racial fusion in this, as any other country, is obviously inter-racial marriage. But there are in this country absolutely no statistics of national scope about the frequency and character of marriages. In a few states, notably New York and Massachusetts, there is official registration (and compilation) of marriage statistics, by much the same mechanism that registers births and deaths. Drachsler (7) has analyzed the New York marriage figures from the point of view of race amalgamation in a notable memoir. But these are exceptional conditions. To know the extent to which the " melting pot " is actually melting we need as the first datum accurate, uniform and complete federal statistics of marriage on an annual basis, and with racial data included.

As these do not exist now, and there is no immediate hope of getting them, we must resort in the present discussion to indirect methods of learning something of the degree to which racial fusion is occurring at the present time. Happily excellent data are at hand for doing this.

Let us begin by defining *an effective marriage within any specified time duration—say one year—as a marriage which produces living offspring within that time period.* Such marriage unions are reproductively effective within that time. This is, of course, the really important consideration. It could possibly make no effect for good or ill to the country if there were a vast number of say Chinese-American marriages, provided all such unions were reproductively absolutely sterile. They would be biologically non-effective marriages, and would not connote the slightest degree of racial fusion. As has already been pointed out, marriage is the first step in racial fusion, but it is *only* the first step. If the marriage does not become reproductively effective there is no true fusion of the races of the contracting parties. So then what, after all, is needed, in addition to straight marriage statistics, is information as to the racial composition of those marriages which at any particular time are *reproductively effective.*

Fortunately data of this latter sort have been regularly published since 1915 for the Birth Registration Area of the United States, collected, checked and compiled by the Division of Vital Statistics of the Bureau of the Census, under the competent direction and supervision of Dr. William H. Davis. Before going into the details as to the character of these statistics it will be well to understand just what is meant by the Birth Registration Area, in order that anyone may judge just what significance is to be attached to its figures, as a sample representing the entire country. In 1919, the year with which we shall deal in the immediately following discussion, the Birth Registration

TABLE II.

Area and population of United States Birth Registration Area in 1919.

United States.		Birth Registration Area.			
		Population.		Land area.	
Population.	Land area (square miles).	Numbers.	Per cent. of total.	Square miles.	Per cent. of total.
104,976,970	2,973,890	61,474,111	58.6	1,075,506	36.2

Area had the geographic and demographic characteristics set forth in Table II. This table is modified from Birth Statistics, 1919, p. 7.

The states included in the Birth Registration Area in 1919 are shown graphically in Fig. 5, which is taken from the frontispiece of Birth Statistics, 1919.

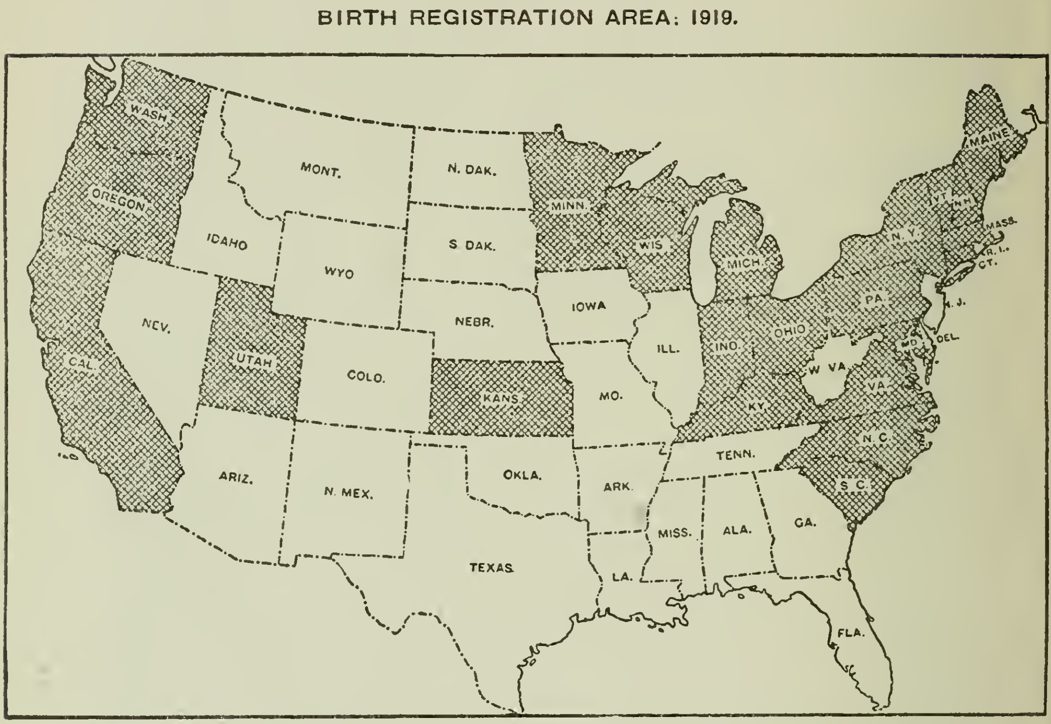

Fig. 5. Map showing states (shaded) in the Birth Registration Area in 1919.

From these data we see that the area in 1919 included nearly six tenths of the entire population of the country, and covered nearly four tenths of the total land area. The Atlantic and Pacific seaboards are practically completely covered, the Middle West is fairly well represented, while the South, the Northwest and the Rocky Mountain regions are practically not represented at all. Probably the most serious omission, from the standpoint of random sampling, is the South. With the reservation which this latter omission implies, we may regard the Birth Registration Area, as constituted in 1919, as fairly representative of the country at large.

TABLE III.

Births (exclusive of stillbirths) of white children, and the country of birth of mother by country of birth of father, in the Registration Area: 1919.

Country of birth of mother.	Country of birth of father.												Totals.
	United States.	Austria (includes Austrian Poland).	Hungary.	Canada.	Denmark, Norway and Sweden.	England, Scotland and Wales.	Ireland.	Germany (includes German Poland).	Italy.	Poland (not specified).	Russia (includes Russian Poland).	Other foreign countries.	
United States	816,546	7,038	1,271	13,033	7,871	8,326	3,788	10,933	11,394	3,634	7,903	8,751	900,488
Austria (includes Austrian Poland)	2,562	46,407	362	34	43	62	22	688	258	615	4,121	599	55,773
Hungary	558	582	14,624	5	8	22	5	307	78	38	313	169	16,709
Canada	11,148	45	7	9,616	111	565	216	121	123	17	65	201	22,235
Denmark, Norway and Sweden	3,583	25	2	110	9,519	140	45	146	32	3	32	222	13,859
England, Scotland and Wales	6,013	92	14	436	143	5,444	459	165	129	23	377	259	13,554
Ireland	5,218	54	4	336	117	702	12,035	140	128	21	29	194	18,978
Germany (includes German Poland)	5,587	465	90	99	132	144	47	6,595	99	216	505	336	14,315
Italy	1,165	65	6	8	8	14	11	11	76,251	8	15	201	77,763
Poland (not specified)	887	274	32	16	11	5	9	97	60	22,562	587	103	24,643
Russia (includes Russian Poland)	2,616	1,670	140	21	25	183	7	310	105	297	53,128	675	59,177
Other foreign countries	3,483	347	73	114	375	204	44	302	503	58	772	30,079	36,354
Totals	859,366	57,064	16,625	23,828	18,368	15,811	16,688	19,815	89,160	27,492	67,847	41,789	1,253,848

In each volume of the Birth Statistics issued by the Bureau of the Census is given a table which shows for each child born the birth nationality, in rather wide groups, of both the father and the mother. This table for 1919 is reproduced here in modified form as Table III. The modifications introduced are: (*a*) male and female births, which are given separately in the original, are here combined, since we are not interested in the sex of the offspring, but only in total marriages effective in 1919; (*b*) the columns and rows of the original table headed respectively "Total foreign," and "Country not stated" are omitted here, as of no purpose in the present inquiry. The net result is that Table III gives all the definitely known facts regarding birth nationality of the partners *in marriages reproductively effective* in 1919 in the Birth Registration Area.

Table III furnishes a picture of the degree of racial assortative mating which was inherent in the marriages which were reproductively effective in 1919 in the Birth Registration Area, or in other words of the degree to which the "melting pot" was melting in that particular year. The data will repay careful and detailed study. One notes at once that the largest frequencies are in those cells which lie on the diagonal of the table connecting the upper left and the lower right corners. These are the cells which exhibit the frequency of effective legitimate matings of like with like in respect of racial stock (in so far as race stock is indicated by nation of birth). Thus there were 816,546 effective marriages in which both male and female were born in the United States: 46,407 in which both members of the pair were born in Austria, and so on. All other cells of the table except those falling on this diagonal theoretically represent racial mixture or fusion of some sort or other. Actually, as we shall presently see, this theoretical assumption is not entirely justified for all the cells. But neglecting this point for the moment in order first to get a broad view, it is plain enough that any coefficient of assortative mating calculated from the table is bound to give a high value. Persons of the same race tend to mate with each other far more than they tend to go outside the race for a mate. The total number of effective marriages included in the table—over one and a quarter millions—is large enough to be impressive.

The next step obviously is to supplement the general impression derived from mere inspection of the table that there is a high degree of racial associative mating (like mating with like), with a more exact determination of its magnitude. The method which at once occurs to one to do this is to calculate by the contingency method the coefficient of racial assortative mating. This I have not done in the present instance for two reasons: first because I know about what the value would be from experience with other tables of racial assortative mating which I have dealt with by the contingency method before. Without correction of non-diagonal, but really like × like cells, the coefficient of contingency will be somewhere in the neighborhood of $+0.8$. The second, and more important reason, is that the coefficient in reality tells very little more than common-sense inspection of the table tells, being in fact merely a single numerical expression of that common-sense result, and gives nothing like so much useful information as does the method of analysis of the material upon which we shall presently embark.

Before doing this I wish to discuss at the outstart one source of error implicit in the table which will be neglected throughout, because its magnitude is so small that it cannot sensibly affect any result, whereas to correct for it would require an inordinate expenditure of time and labor, even if, with the available data, it ever could be fully corrected. The error is this. I have referred throughout to the frequencies of Table III as frequencies of "effective marriages." Actually, of course, they are frequencies of births. Not all births are single births. Some are twins, triplets, etc. In the case of twins the *same* marriage gets counted in Table III as effective twice in 1919. So, of course, it was in fact from the standpoint of population growth. But from the standpoint of analyzing the racial fusions involved it apparently implies one more pair of parents of the particular sort of racial combinations involved than in fact existed. The number of multiple births, however, in proportion to total births, is so small as to make this a negligible source of error in any conclusions which will here be drawn from the figures. Thus in 1919 there were 12.2 cases of plural births per 1000 mothers of that year. Or, put in another way, there were only 32,204 live infants born plurally, in a total of 1,373,438 live infants born in 1919.

The first problem on which interest centers may be stated in this way: what proportion of the marriages effective in adding to the population in 1919, were racially pure (race as always being indicated by country of birth), as compared with those which were racially mixed, the mixing being either (*a*) of American-born with foreign-born, or (*b*) of various different sorts of foreign-born together?

To get a first approximation to the answer to this question Table IV has been prepared from Table III and similar data in Birth Statistics, 1919, showing the results for the Registration Area as a whole and for each state separately. The columns contain the following data:

Column A.—The sum of the diagonal cells of Table III (and similar tables for each state). The entries for this column show the total number of births from effective marriages in which both members of the pair were born *in the same country,* and were thus inferentially of the same racial stock. As has already been pointed out this inference is, in a broad way, justified, except for the United States. There we step over into another "universe of discourse." Even there the two members of the married pair are at least alike in these respects, that: (1) they were both born in the United States, and (2) they have been subjected to Americanizing influences for an average period of from 28 to 33* years. This means that they went, in general, to American schools and were subjected to Americanizing influences throughout the most formative period of life. Although real racial differences are not to be wiped out in any such easy way or short period as this, still no one who has attentively observed our foreign peoples will deny that the individual born and reared in this country is in many respects definitely different from the foreign-born immigrant, coming at any age after say 12 to 15. For the present then, we shall treat the U. S. × U. S. effective marriages as though they were directly comparable to the other diagonal cell groups; reverting later to further discussion of the point.

Column B.—This column contains the sum of the frequencies in the cells forming the first *row* of Table III, *exclusive* of the first cell in the row (U. S. × U. S.). The figure thus shows how many effective marriages in 1919 there were in the B. R. A.,* in which the *female* member of the pair was American born. This will, in general, indicate the extent of the assimilation of foreign males by the American females of the existing population.

Column C.—This contains the sum of the frequencies in the cells forming the first *column* of Table III, again exclusive of the topmost cell (U. S. × U. S.). This is the counterpart of Column *B,* showing the extent of effective fusion of American-born male elements with foreign-born females. Columns *B* and *C* together show the whole extent of the effective fusion in the B. R. A. in 1919 of foreign with native-born elements.

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* These figures are respectively about the average ages of mothers and fathers in 1919.

* This will be used as a convenient abbreviation of Birth Registration Area throughout the remainder of the discussion.

Column D gives the sums of the figures in Columns *B* and *C* for the purpose above indicated.

Column E gives the total number of effective marriages in which both partners were born in the United States. This is the frequency in the upper left-hand corner cell of Table III, and the corresponding cell for the state tables.

Column F gives the total frequency in all the diagonal cells of Table III, *exclusive* of the corner cells dealt with in Column *E*. In other words these figures show the total number of racially *pure* effective marriages in which both partners were foreign-born.

Column G contains the sum of the frequencies in all those cells of Table III which *are not included* in Columns *A*, *B*, and *C* of Table IV. These are the cells which fall neither upon the diagonal, nor upon the bordering row or column of the table. The summed frequencies represent the total number of effective marriages in which racial mixture or fusion occurred, with neither partner in the union American-born. The figures show the extent to which the several foreign elements in our population are failing to keep racially pure, without at the same time mixing with American-born.

Column H gives the sums of the entries in Columns *F* and *G*. These figures show the total numbers of effective marriages in the B. R. A. in 1919 in which both members of the united pair were of foreign birth.

Column I gives the percentage which each entry in Column *D* is of the corresponding entry in Column *A*. In other words the figures in Column *I* answer the following question: what proportion do the effective marriages which represent a fusion or "melting" of foreign stocks *with American-born elements* form of the effective marriages in which like mated with like racially (counting U. S. × U. S. as a like mating)?

Column J gives the percentages which the several entries in Column *D* are of the corresponding entries in Column *E*. These figures show the proportions which effective fusions of native-born with foreign-born elements form of effective matings of native-born with native-born. This column may be taken to indicate the rate at which true net amalgamation is taking place.

Column K gives the percentages which the several entries in Column *D* are of the corresponding entries in Column *F*. The figures show the relative tendencies of foreign stocks to mate (effectively) with native-born stocks, as compared with their tendency to mate (effectively) within their own racial groups in this country.

Column L gives the percentage which each entry in Column *F* is of the corresponding entry in Column *E*. The figures show the proportions of racially pure effective matings of foreign-born as compared with native-born elements.

Column M gives the percentage which each entry in Column *G* is of the corresponding entry in Column *F*. The figures indicate the relative tendency of the foreign-born stocks to mate (among themselves) pure, as compared with their tendency to mate with other foreign-born stocks.

Column N gives the percentage which each entry in Column *H* is of the corresponding entry in Column *E*. The figures compare relatively the effective results of matings in which both parties are foreign-born with those matings in which both partners are native-born.

The several states in Table IV are arranged in descending order of the entries in Column *I*.

In the discussion of Table IV it will be well to confine the attention in the first instance to the percentage columns beginning with *I*, noting only that the total number of effective marriages dealt with, over a million, is large enough to command respect. From the table the following points are especially to be noted.

1. For the entire B. R. A. the percentage of amalgamation or fusion of foreign-born stocks with native-born, as compared with racially like effective matings of all sorts, as indicated by effective marriages in 1919, is just under 11.5 per cent. That is, for about every ten effective marriages in which the partners were of like nativity there was one in which one partner was of American and the other of foreign nativity. On the whole this appears a reasonably large proportion, when one considers inherent prejudices which must be overcome before such marriages can occur. Of course one realizes fully that some unknown, but certainly significant proportion of the 126,762 American × foreign-born cross matings are really not *racially* cross at all but like, one partner merely having been born in the United States. But fully granting this, there are two further points to be noted, namely: first, that American birth and upbringing to marrying age tend to create a considerable measure of antipathy or

TABLE IV

Showing for the B. R. A., and each separate state certain relations of effective marriages in 1919.

Area.	*A* All like × like matings.	*B* Native ♀♀ × foreign ♂♂.	*C* Native ♂♂ × foreign ♀♀.	*D* Sum of *B* and *C*.	*E* Native ♂♂ × native ♀♀.	*F* All foreign pure matings.	*G* All foreign mixed matings.	*H* Sum of *F* and *G*.	*I* Per cent. which *D* is of *A*.	*J* Per cent. which *D* is of *E*.	*K* Per cent. which *D* is of *F*.	*L* Per cent. which *F* is of *E*.	*M* Per cent. which *G* is of *F*.	*N* Per cent. which *H* is of *E*.
Total B. R. A.	1,102,806	83,942	42,820	126,762	816,546	286,260	24,280	310,540	11.49	15.52	44.28	35.06	8.48	38.03
New Hampshire	6,689	996	788	1,784	4,317	2,372	129	2,501	26.67	41.32	75.21	54.95	5.44	57.93
Maine	12,196	1,545	1,448	2,993	9,888	2,308	109	2,417	24.54	30.27	129.68	23.34	4.72	24.44
Massachusetts	67,190	8,217	6,915	15,132	31,468	35,722	2,437	38,159	22.52	48.09	42.36	113.52	6.82	121.26
Minnesota	40,920	6,566	2,625	9,191	33,028	7,892	705	8,597	22.46	27.83	116.46	23.89	8.93	26.03
Washington	19,416	2,455	1,244	3,699	16,412	3,004	458	3,462	19.05	22.54	123.14	18.30	15.25	21.09
Michigan	68,161	7,909	4,080	11,989	50,114	18,047	1,913	19,960	17.59	23.92	66.43	36.01	10.60	39.83
California	42,293	4,888	2,169	7,057	31,333	10,960	1,039	11,999	16.69	22.52	64.39	34.98	9.48	38.30
Wisconsin	45,692	5,277	2,118	7,395	38,329	7,363	678	8,041	16.18	19.29	100.43	19.21	9.21	20.98
Vermont	5,910	551	401	952	4,923	987	63	1,050	16.11	19.34	96.45	20.05	6.38	21.33
Connecticut	27,638	2,663	1,566	4,229	11,468	16,170	1,151	17,321	15.30	36.88	26.15	141.00	7.12	151.04
New York	183,463	17,983	9,202	27,185	93,947	89,516	9,578	99,094	14.82	28.93	30.37	95.28	10.70	105.48
Oregon	11,290	994	513	1,507	10,284	1,006	192	1,198	13.35	14.65	149.80	9.78	19.09	11.65
Utah	11,216	810	611	1,421	10,304	912	103	1,015	12.67	13.79	155.81	8.85	11.29	9.85
Pennsylvania	176,840	13,029	4,789	17,818	117,171	59,669	3,541	63,210	10.08	15.21	29.86	50.92	5.93	53.95
District of Columbia	5,255	300	174	474	4,736	519	66	585	9.02	10.01	91.32	10.96	12.72	12.35
Ohio	99,556	5,067	2,206	7,273	79,342	20,214	1,305	21,519	7.31	9.17	35.98	25.48	6.46	27.12
Maryland	25,347	1,084	535	1,619	22,579	2,768	302	3,070	6.39	7.17	58.49	12.26	10.91	13.60
Kansas	33,180	1,255	530	1,785	31,493	1,687	97	1,784	5.38	5.67	105.81	5.36	5.75	5.66
Indiana	55,027	1,454	566	2,020	51,000	4,027	283	4,310	3.67	3.96	50.16	7.90	7.03	8.45
Virginia	40,334	395	159	554	39,703	631	70	701	1.37	1.40	87.80	1.59	11.09	1.77
Kentucky	52,820	312	112	424	52,532	288	43	331	.80	.81	147.22	.55	14.93	.63
South Carolina	21,681	84	24	108	21,586	95	10	105	.50	.50	113.68	.44	10.53	.49
North Carolina	50,692	108	45	153	50,589	103	8	111	.30	.30	148.54	.20	7.77	.22

prejudice towards the more recent immigrant of the same race. In making this statement the writer is relying on his own personal observations of foreign stocks in our country. In many cases the American-born child of foreign parents desires passionately to be a "pure American," to slough off and forget the attributes which characterize, as he or she thinks, the "wop," using this as a generic term of disparagement for the foreigner. Un-American language, names and customs of all sorts, excite in many cases a positively phobic reaction in the native-born children of foreign-born parents. How unfortunate this all is is only too often apparent. The American substitutes for the dropped foreign habits are frequently poor substitutes indeed. The ignorant and foolish child assimilates the worst of American habits and manners, while letting go of things often distinctly fine and precious.

In the second place it must be remembered that in the 816,546 effective marriages of native-born there are many racially unlike matings. Perhaps this balances off in good part the error inherent in cross-mating figures.

2. Thirteen states exhibit a fusion percentage higher than the average, with New Hampshire followed by Maine at the top, with approximately one quarter of all the effective marriages between native- and foreign-born partners. In these two states the vast majority of these mixed marriages includes a French Canadian as one of the partners. The group above the average in fusion percentage includes all the New England States except Rhode Island (which is not in the B. R. A.) and New York. This would be reasonably expected. These are old states in which (*a*) there has been time for the more superficial and frothy aspects of race prejudice to evaporate, and (*b*) the foreign population has, by and large, been of rather distinctly superior quality. In the Middle West, Minnesota, Michigan, and Wisconsin give percentages well above the average. Here again superior foreign-born elements have been long in residence and demonstrated their worth. From the Far West, Washington, California, Oregon and Utah give percentages more or less above the average. Perhaps this is to be accounted for by the rather distinctly open-minded racial tolerance of these communities, so long as Orientals are not involved.

3. The distinctive feature of the group of states giving fusion percentages below the average is that it is outstandingly a southern group. Seven out of the ten states in the group lie south of the Mason-Dixon line. This is probably to be accounted for by two

factors: (*a*) an intense prejudice against racial miscegenation in general, founded upon the concrete case of the negro, and (*b*) a relatively small proportion of foreign-born in the communities concerned.

4. This brings up a question of obvious interest. When the states are arranged in descending order of effective race fusion percentages as in Table IV (from Column *I*), is not this also about the same order in which they would stand in respect of percentage of foreign-born white populations? Table IV shows that this is in fact the case to a high degree of approximation.

TABLE IV *a*.

Comparing percentages of foreign-born whites in the total population in 1910 with Column I of Table IV.

States.	Column *I*. Table IV, fusion percentage.	Per cent. of foreign-born whites in population in 1910.
New Hampshire	26.67	22.4
Maine	24.54	14.8
Massachusetts	22 52	31.2
Minnesota	22.46	26.2
Washington	19.05	21.1
Michigan	17.59	21.2
California	16.69	21.8
Wisconsin	16.18	22.0
Vermont	16.11	14.0
Connecticut	15.30	29.5
New York	14.82	29.9
Oregon	13.35	15.3
Utah	12.67	17.0
Pennsylvania	10.08	18.8
District of Columbia	9.02	7.4
Ohio	7.31	12.5
Maryland	6.39	8.0
Kansas	5.38	8.0
Indiana	3.67	5.9
Virginia	1 37	1.3
Kentucky	.80	1.7
South Carolina	.50	0.4
North Carolina	.30	0.3

How close the parallelism is between the two columns of Table IV *a* is shown graphically in Fig. 6.

The important fact strikingly demonstrated in Table IV *a* and Fig. 6 is that the amount of racial amalgamation or fusion going on in the several parts of the American " melting pot " is proportional in the most direct and close way to the amount of foreign-born white stock in the local population. In other words the dominant and outstanding factor in determining whether there shall be true effective assimilation of foreign elements into the established American population is simply the opportunity afforded by propinquity—which is statistically one of the most potent factors in bringing about any and all marriages. Given a community in which there are many foreign-born whites as more or less settled residents, there will be relatively many marriages in which one of the partners will be of American and the other of foreign nativity.

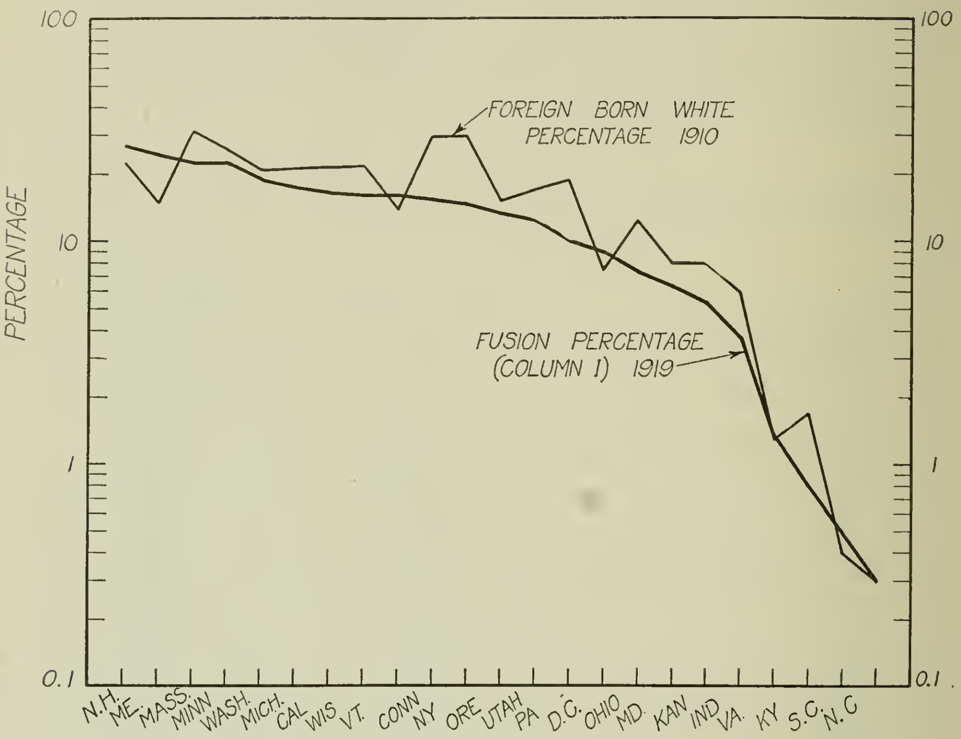

FIG. 6. Showing the close agreement which exists between the percentages of mixed marriages of native and foreign-born effective in 1919, and the percentages in the population of foreign-born whites in 1910.

5. Turning to Column *J* of Table IV, which gives the percentage which the total number of reproductively effective cross-matings, in

which one partner was American-born, is of the number of effective matings in which both partners are native-born, it is seen that while the percentages are, as must necessarily be the case, somewhat higher than those of Column *I,* they nevertheless follow about the same order. Some of the individual entries are of interest. Thus in the case of Massachusetts there were nearly one half (48.09 per cent.) as many matings effective in producing offspring in 1919 in which fusion of American- and foreign-born occurred, as there were effective matings where both partners were native-born. Just as in the preceding case it is evident that opportunity is the important factor in making the melting pot melt. Figure 7 is of the same general character as Fig. 6 except that here the *J* Column percentages, which may be taken as the closest approximation to actual, net, effective racial amalgamation, are plotted against percentages of foreign-born whites in 1910.

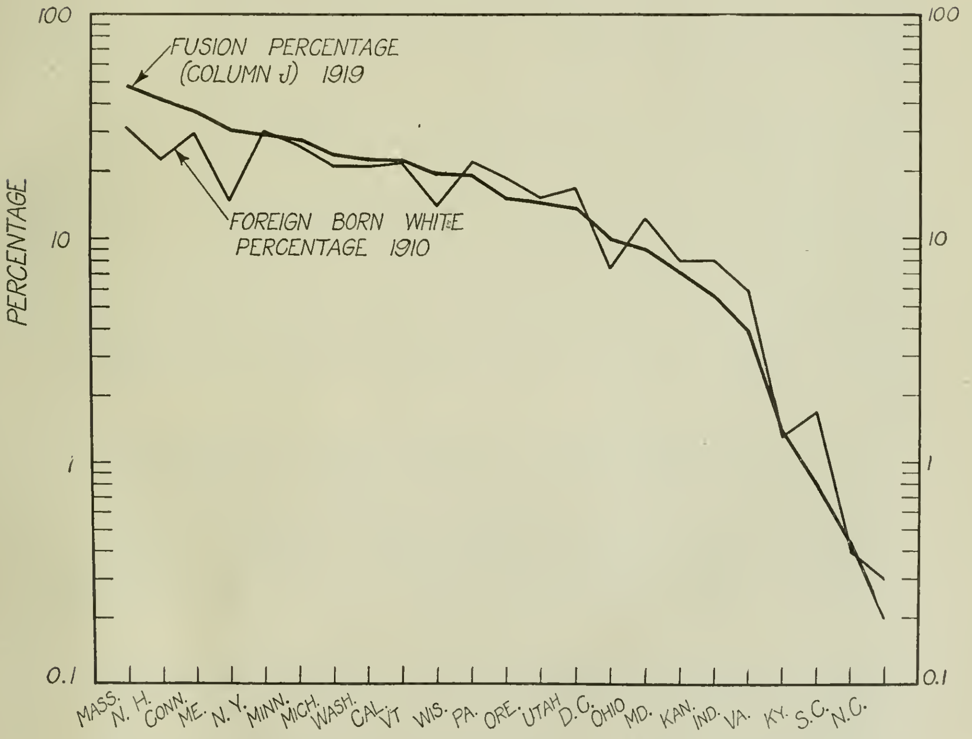

FIG. 7. Showing the agreement between percentages of mixed American and foreign effective marriages in 1919 (percentages on native × native base only), and percentages of foreign-born whites in the population in 1910.

The agreement between the two lines is even closer in this diagram than was the case in Fig. 6. There can be no doubt that where the most foreign-born whites are, there occurs the most mixed mating with native-born, not only absolutely but also relatively, in proportion both to all racially pure matings and to purely American (i.e., native × native) matings.

And there can be no doubt, under the circumstances, about the reality of racial fusion in this country. When from one fourth to one half as many children are produced in a given year from mixed matings (in which one partner is American) as from matings of strictly American-born persons, amalgamation of the immigrant elements into the previously existing complex is certainly going on apace.

6. Column K gives us a view of the same phenomenon from another angle. Here the question presented is: what proportion do effective mixed matings (in which one partner is American-born) form of racially pure matings in the same communities in which both partners are of foreign birth? In ten out of the twenty-three states there are *more* effective mixed matings of the amalgamating type—or, if one chooses, of the Americanizing type—than there are of racially pure foreign × foreign matings. These are the states exhibiting percentages greater than 100 in Column K. A better appreciation of the essential point here can be gained by a graphic presentation. This is given in Fig. 8, where the data of Column K are plotted against the percentages of foreign-born whites in 1910. In the diagram the states are arranged in descending order of proportion of foreign-born whites in 1910 (heavy line). This, it will be noticed, is the opposite of the plan in Figs. 6 and 7, where these data were given as the light instead of the heavy line.

It is at once apparent that, in general, there is no significant association between the two variables here plotted. Leaving out of account for the moment the three states with the highest percentage of foreign-born whites, namely, Massachusetts, New York and Connecticut, it is clear that the remaining 20 states proceed across the diagram in what amounts to a horizontal line, in respect of the proportion of fusion to pure all-foreign matings, while at the same time the percentage of foreign-born in the population is steadily decreasing. In the eastern states mentioned the case is different. The proportion of foreign-born whites in total population is there highest. The proportion of fusion matings to pure foreign-born matings is practically the lowest. Here we see the effect of the condensed, homo-

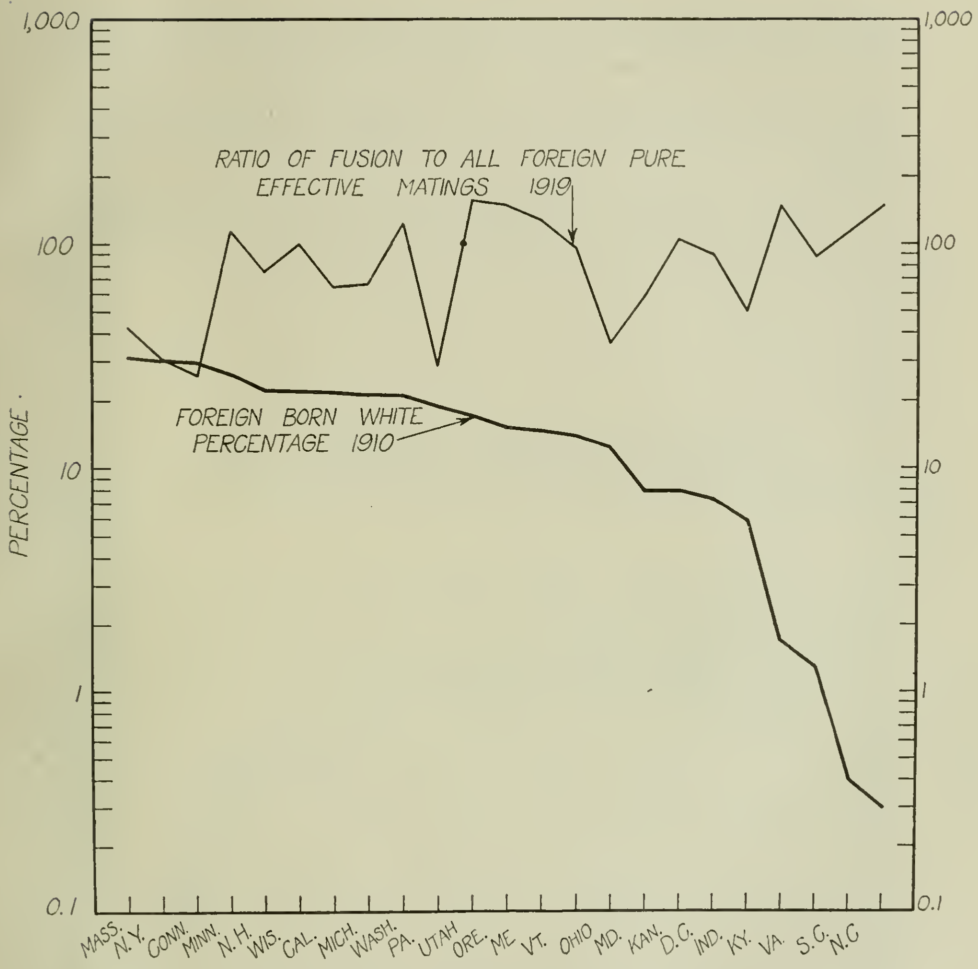

Fig. 8. Showing the relation between (*a*) the proportion of foreign-born whites in the population in 1910, and (*b*) the percentage which "fusion" effective matings (as defined in text) are of effective all-foreign pure matings in the same communities.

geneous "foreign colony" type of population, flourishing in great industrial centers. Yet the fact must not be overlooked that all three of these states, Massachusetts, Connecticut and New York, are above the average in the percentage which fusion matings form of pure American-born matings (cf. Column *J*). There is no contradiction in these two facts, though at first glance there might appear to be. The facts merely mean that in each of these three commonwealths there is a comparatively large, well-settled, foreign-born population, reproducing at a relatively much more rapid rate, both pure and when fused with American-born stock, than is the strictly American-born stock by itself.

7. Column *L* gives for each state the percentage which effective foreign-born × foreign-born matings form of the effective native-born

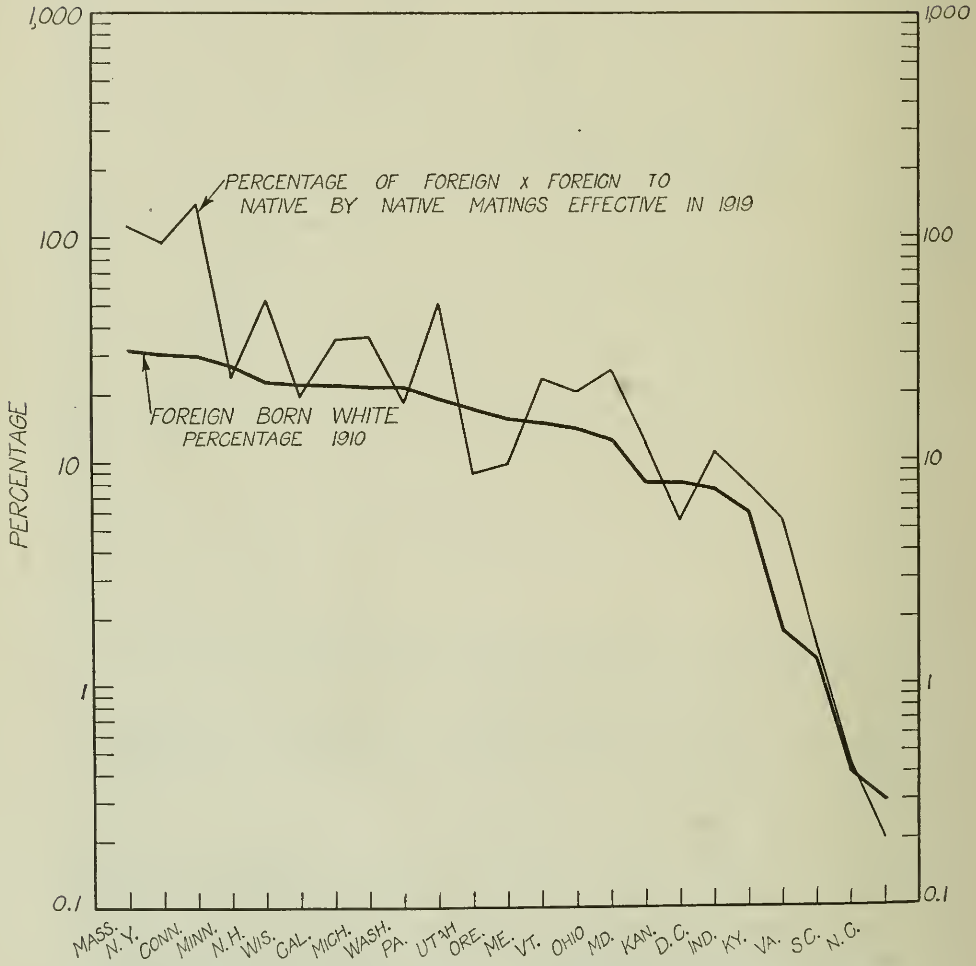

FIG. 9. Showing the relation between (*a*) percentage of foreign-born in total population in 1910 (heavy line), and (*b*) proportion of foreign × foreign matings to native matings effective in 1919.

× native-born matings. Plotting these results against the percentage of foreign-born in the total population in 1910 gives the result shown in Fig. 9.

It is evident that the two curves run generally together, meaning that the proportion of foreign × foreign effective matings tends to be highest where there are proportionally the greatest number of foreign-born in the population, and lowest where there are the fewest. But there are some striking outstanding points, which merit special consideration. In the three states Massachusetts, New York and Connecticut there were actually, in 1919, as many or more matings of the type foreign-born × foreign-born, as there were of the type native-born × native-born, *effective in producing offspring*. This means, put in another way, that in these three states as large or larger additions to the future population were made in 1919 by the foreign-born as by the native-born. Furthermore in three other states, namely New Hampshire, Pennsylvania and Maine, the effective foreign × foreign matings were more than half as numerous as the effective native × native.

There can be no doubt that, on the average, by whatsoever standard measured, the native-born are "Americanized" in higher degree than the foreign-born. It would appear that in the six states named, the less Americanized elements are multiplying too fast in proportion to the more Americanized. There can furthermore be no doubt that this state of affairs arises in largest degree as a consequence of the crowding of large, purely foreign "colonies" in great industrial centers of population. This is certainly true of Massachusetts, New York, Connecticut and Pennsylvania. In Maine and New Hampshire the same mechanism essentially is at work and is the *causa princeps* of the result, but it operates chiefly on a different sort of foreigner, namely the French-Canadian, about whose eventual and satisfactory Americanization no particular doubt can be felt. In the other four states named, what appears to be needed chiefly is to break up the too close geographical segregation. Then the problem will take care of itself. This appears to me to be abundantly proved by the results which have already been presented here. Give the foreign elements a chance to meet and really know the native population, and the melting pot promptly begins to seethe and bubble. The blame for the sort of conditions depicted by the statistics of Massachusetts, New York, Connecticut and Pennsylvania, in my judgment, lies far heavier upon the native Americans than upon the foreigners. Similarly situated we should all act much as the foreigners do in respect of our mating habits.

8. Column *M* presents another angle of the problem. It shows the percentage which effective all-foreign mixed matings are of effective all-foreign pure matings. The all-foreign mixed matings are those in which both partners are foreign-born, but the husband has a different country of birth than the wife. In other words we have in this column a measure of the extent to which assortative mating in respect of race occurs among the foreign-born in this country, excluding all mixtures of foreign with American-born. It is seen at once that all percentages are relatively small, the largest being 19.09 in the case of Oregon. The comparison between the entries of Column *M* and those of Column *K* is highly significant. The foreigner in this country is much more likely to marry an American-born person, if he does not marry one of his own race, than he is to marry some other foreigner not of his own race. This fact would become even more emphasized if we were able to make the proper corrections for Jews of different countries of birth.

9. Column *N* compares the total of effective matings of foreign × foreign-born (whether pure or mixed) with the total of native × native effective matings. In three states, Massachusetts, New York and Connecticut, these percentages are over 100, as would of course be expected from what has gone before. In these states the foreign-born produced more children in 1919 than did the native-born. But, after all, the *children* were native-born and will be subjected to Americanizing influences from birth onwards.

Up to this point we have been considering very broad types of matings, and examining the extent of effective fusion of races in different parts of the B. R. A. It will now be well to examine the facts from another angle, and from one point of view summarize them.

Table V shows that in 1919 there were in the B. R. A. 78 racially different types of mating effective in producing offspring, not separating sexes of either mated partners or offspring. These matings are given in descending order of number of children produced in Table V. It is to be understood, here as before, of course, that " race combination " merely means " so far as indicated by country of birth," and is in so far and in that sense not accurate.

From Table V it is seen that 65 per cent. of the children produced in the B. R. A. in 1919 were from American-born parents. Of the remaining 35 per cent. the make-up was as follows: 10.113 per cent.

TABLE V.

Net effectiveness in producing next generation.

Order.	Race combination.	Number of births.	Per cent.	Order.	Race combination.	Number of births.	Per cent.
1	U. S.×U. S.	816,546	65.123	30	Austria×Hungary	944	.075
2	Italy×Italy	76,251	6.081	31	Austria×Poland	889	.071
3	Russia×Russia	53,128	4.237	32	Poland×Russia	884	.071
4	Austria×Austria	46,407	3.701	33	Germany×Russia	815	.065
5	Other foreign×other foreign	30,079	2.399	34	Italy×other foreign	704	.056
6	U. S.×Canada	24,181	1.929	35	Germany×other foreign	638	.051
7	Poland×Poland	22,562	1.799	36	Scandinavia×other foreign	597	.048
8	U. S.×Germany	16,520	1.318	37	U. K.×Russia	560	.045
9	Hungary×Hungary	14,624	1.166	38	Canada×Ireland	552	.044
10	U. S.×U. K.	14,339	1.144	39	U. K.×other foreign	463	.037
11	U. S.×Italy	12,559	1.002	40	Hungary×Russia	453	.036
12	U. S.×other foreign	12,234	.976	41	Hungary×Germany	397	.032
13	Ireland×Ireland	12,035	.960	42	Austria×Italy	323	.026
14	U. S.×Scandinavia	11,454	.914	43	Canada×other foreign	315	.025
15	U. S.×Russia	10,519	.839	44	Germany×Poland	313	.025
16	Canada×Canada	9,616	.767	45	U. K.×Germany	309	.025
17	U. S.×Austria	9,600	.766	46	Scandinavia×U. K.	283	.023
18	Scandinavia×Scandinavia	9,519	.759	47	Scandinavia×Germany	278	.022
19	U. S.×Ireland	9,006	.718	48	Hungary×other foreign	242	.019
20	Germany×Germany	6,595	.526	49	Ireland×other foreign	238	.019
21	Austria×Russia	5,791	.462	50	Canada×Scandinavia	221	.018
22	U. K.×U. K.	5,444	.434	51	Canada×Germany	220	.018
23	U. S.×Poland	4,521	.361	52	Ireland×Germany	187	.015
24	U. S.×Hungary	1,829	.146	53	Scandinavia×Ireland	162	.013
25	Russia×other foreign	1,447	.115	54	Poland×other foreign	161	.013
26	U. K.×Ireland	1,161	.093	55	Austria×U. K.	154	.012
27	Austria×Germany	1,153	.092	56	U. K.×Italy	143	.011
28	Canada×U. K.	1,001	.080	57	Ireland×Italy	139	.011
29	Austria×other foreign	946	.075				

Order.	Race combination.	Number of births.	Per cent.	Order.	Race combination.	Number of births.	Per cent.
58	Canada×Italy. . .	131	.010	69	Scandinavia×Italy.	40	.0032
59	Italy×Russia. . . .	120	.0096	70	Hungary×U. K. .	36	.0029
60	Germany×Italy. .	110	.0088	71	Ireland×Russia. .	36	.0029
61	Canada×Russia. .	86	.0069	72	Canada×Poland .	33	.0026
62	Hungary×Italy. .	84	.0067	73	Ireland×Poland. .	30	.0024
63	Austria×Canada.	79	.0063	74	U. K.×Poland. . .	28	.0022
64	Austria×Ireland .	76	.0061	75	Scandinavia×Poland.	14	.0011
65	Hungary×Poland.	70	.0056	76	Hungary×Canada.	12	.00096
66	Austria×Scandinavia. . .	68	.0054	77	Hungary×Scandinavia. . .	10	.0008
67	Italy×Poland. . . .	68	.0054	78	Hungary×Ireland.	9	.0007
68	Scandinavia×Russia.	57	.0045				
					Total.	1,253,848	100.004

of the total children had one parent native-born, leaving approximately 25 per cent. of the total births having both parents foreign-born. So then it appears that almost exactly 75 per cent. of the total children had one or both parents native-born. Of the foreign-born effective matings the highest percentage is exhibited by the Italians, next by the Russian born, next by the Austrian born, and next by the residue group "other foreigners." These four foreign groups contributed together some 16 per cent. of the total children. It is probably conservative to estimate that one half at least of these parental groups were Jewish. The first twelve of the matings in the table produced over 90 per cent. of the children born in the B. R. A. in 1919. Whether the above facts, which are shown graphically in Fig. 10, will be regarded as alarming or reassuring depends upon one's preconceived point of view. From one point of view it will be regarded an evil of alarming proportions that but 65 per cent. of children born in this country have both parents native-born. But, on the other hand, as the figures show, the proportion having both parents native-born is overwhelmingly larger than that for any other mating. Our present task, however, is simply to present the facts, to as great a degree of approximation as is possible.

Throughout the discussion of this point we have been compelled, by lack of the raw statistical data necessary to do anything else, to take country of birth as indicative of race. Everyone knows that it is not accurately so. It is at best only an approximation, and conceals

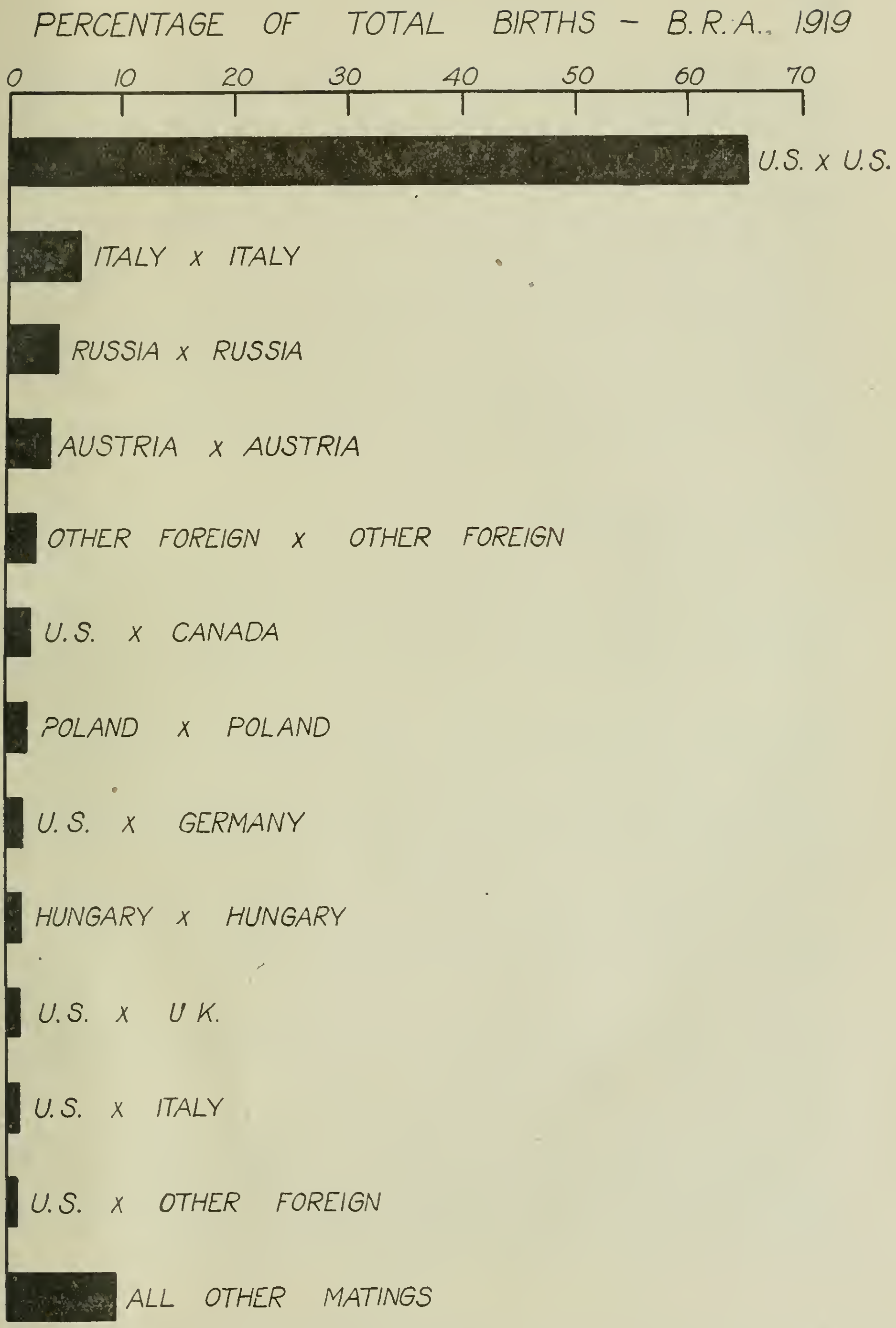

Fig. 10. Bar diagram showing proportion of children produced in the B. R. A. in 1919, from different types of matings.

many pertinent facts. Again, because of the lack of the necessary data, it is impossible to make any correction of Tables III, IV and V which will, after the expenditure of a considerable amount of time that would be involved in attempting corrections, tell us anything more exact than what we know to be the broad features of the case. The errors in Tables III, IV and V incident to taking birthplace as an index of race are, broadly speaking, as follows:

1. An error which probably tends to make the degree of racial assortative mating in effective marriages appear *lower* than it really is, is found in the fact that the U. S. × U. S. group contains many different racial elements. Many of these probably mate assortatively and should, on a strictly racial basis, be taken out of the upper left hand corner cell of Table III and distributed to their proper diagonal cells down the table. But others of this sort do not mate in strict racial assorting, and they should be distributed to the appropriate cells of the table off the diagonal.

2. Another factor of much greater importance in lowering the apparent below the true degree of racial assortative mating, is found in the cells not on the diagonal in Table III with disproportionately high frequencies; as for example, U. S. ♂ × Russian ♀ (frequency 2616), Austrian ♂ × Russian ♀ (frequency 1670), etc. Now while these appear to be cross-matings, as a matter of obvious fact, they are in the vast majority of cases strictly pure matings, namely of Jewish ♂ × Jewish ♀. They should in some unknown, but certainly large proportion, be transferred to the diagonal cells. Another example of the same thing, but not involving Jews, is seen in the disproportionately high frequencies in the cells Canadian ♂ × U. K. ♀ (frequency 436) and U. K. ♂ × Canada ♀ (frequency 565). What we really have, in a great proportion of these cases, is the mating of Canadian-born English, Welsh or Irish males with foreign-born females *of the same race.*

The first of the sources of error named I am personally inclined largely to discount, because of the conviction that about the only logically tenable simple definition of an American, in the sense the word is here used, is that of nativity. An American is a person born in the United States.* The only *racially* pure American is the Amer-ind. Everybody else is something other than American racially. I can not see logically but that a person born in the United States in 1920 has just as much right to call himself an American as one born here in 1620. And further this right is just as logically passed on hereditarily now as it was then. In other words is there any reason not to accept little Gaetano Tomasino (let us say) as a friend and brother citizen, though his ancestors first landed on these fair shores in the early spring of 1920 instead of the late autumn of 1620, *provided,* and just as long as, *Gaetano behaves like an American?* If and when he does not then plainly something must be done about it. Practically, and in fact, that is the way in which Americans of all varieties, including that odd and rather dangerous creature now so much to the fore, the "100 per cent. American," have come into being.

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* I realize as fully as my Canadian friends the unfortunate element in the common usage of "American" with reference solely to the United States rather than to North America. Canadians are of course as much Americans as we are. But, in point of fact, because United States is not a term easily put into

Divorce.

Divorce is unquestionably a factor in the biology of human reproduction, and should therefore be considered in any general resumé of the subject. From the point of view of the present discussion, however, it is wholly impossible to do anything with this topic, since no data are available. In "Marriage and Divorce, 1916," a special report of the Bureau of the Census issued in 1919, the following statement is made:

> Two investigations with respect to marriage and divorce have been made previously by the Federal Government—one in 1887–88 by the Department of Labor, covering 20 years from 1867 to 1886, inclusive, and the other, in 1906–7 by the Bureau of the Census, covering the 20 years from 1887 to 1906, inclusive. These two investigations together covered a consecutive period of 40 years.

The results of the first of these studies are incorporated in the second. It thus results that the entire statistical knowledge of marriage and divorce for this country as a whole is contained in these volumes. And in no one of these are there any data about race or nationality. Consequently from our present viewpoint nothing can be done with the material.

IV.

NATALITY.

The most important data regarding births, from the viewpoint of the present discussion, have already been presented in the section

adjective form, while America is, common usage has seized upon the last word of the official designation of this country rather than the first two, to use as the descriptive adjective for her citizens.

on marriage, as a result of dealing with *effective* marriages solely. It remains here to consider certain special topics not there dealt with. These topics are (*a*) fertility, (*b*) illegitimacy, (*c*) still-births.

A. Fertility.

The general fact that foreign-born women are much more fertile, under American conditions, than native-born is well known, and can be shown in many different ways. Perhaps the most striking way to set forth the broad fact is to show the relation of births to married women of child-bearing age in the population as a whole. This is done in Table VI, which is in rearranged form, a table given on p. 10 of Birth Statistics, 1919.

TABLE VI.

Showing the relation between the number of foreign-born women of child-bearing age and their contributions to the population.

State.	Per cent. of			
	Children of foreign-born mothers to total children of white mothers.			Foreign-born married females 15–44 to white married females 15 to 44.
	1919.	1918.	1917.	1910.
Rhode Island	†	53.59	55.76	49.94
Massachusetts	52.58	53.20	55.36	48.87
Connecticut	56.76	58.20	60.21	46.36
New York	48.99	49.10	51.64	42.71
Minnesota	21.90	22.25	24.86	33.99
New Hampshire	37.78	38.51	40.81	32.69
Pennsylvania	33.88	34.52	36.33	27.77
Michigan	29.11	30.08	32.46	26.45
Wisconsin	18.70	19.08	20.62	26.40
California	28.04	*	*	23.74
Washington	19.86	20.16	21.69	23.24
Maine	25.13	24.24	25.92	21.89
Utah	12.73	13.17	14.56	18.83
Vermont	20.71	21.38	22.47	18.11
Ohio	21.71	21.87	23.11	15.51
Oregon	12.98	*	*	14.81
Maryland	13.15	13.16	14.29	13.11
Kansas	6.54	6.12	6.91	8.12
Indiana	8.43	8.46	8.95	5.72
Virginia	2.06	2.06	2.05	2.35
Kentucky	0.82	0.77	0.86	1.55
South Carolina	0.58	*	*	0.90
North Carolina	0.30	0.23	0.34	0.47

* Not added to registration area until later date.

† Excluded because all transcripts of birth certificates for the year were not received.

From Table VI it is seen that in 12 of the 23 states in the table the foreign-born women were the mothers of a higher percentage of children than would be expected if they were no more fertile than the native-born women of the same age groups. In the case of Connecticut the percentage of births rises to 10 or more points higher than the percentage of representation in the population.

For one state, New York, Eastman (8) has made a very careful and thorough study of the birth-rates by race. Table VII shows some of his chief results.

TABLE VII.

Births to white mothers, according to nativity of mothers, in New York State, (exclusive of New York City) in 1916. (After Eastman.)

Nationality of mother.	Estimated population in 1916.	Number of births.	Number of births per 1,000 estimated population.	Crude birth rate of native country according to last report before war.	Date of last report before the war.
Total white	4,643,786	*102,834	22.1	....	....
Native white	3,777,685	64,889	17.2	....	....
Foreign-born white	866,101	37,914	43.8	....	....
English, Scotch, and Welsh	97,695	1,869	19.1	‡24.1 §25.5	1913
Irish	124,467	1,879	15.1	22.8	1913
German (includes German Poles)	171,435	2,421	14.1	27.5	1913
Italian	141,845	12,998	91.6	31.7	1913
Russian (includes Finland and Russian Poland)	82,195	7,281	88.6	44.0	1909
Austro-Hungarian (includes Austrian Poles)	81,256	7,307	89.9	||31.3 ¶36.3	1912
Canadian	104,270	2,219	21.3	†24.0	1913
Other foreign-born	62,938	1,940	30.8	....	....

Commenting on this table Eastman says:

> The features of interest that at once attract the attention are the extraordinarily high rates of the Italians, Russians and Austro-Hungarians (which amount to from 100 per cent. to almost 200 per cent. higher here than in the mother countries), and the exceptionally low rates of the Irish and Germans. A study of the statistics of immigration as contained in the reports of the Com-

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* Nativity of mother unknown in the case of 31 births.

† Province of Ontario.

‡ England and Wales.

§ Scotland.

|| Austria.

¶ Hungary.

missioner General of Immigration of the United States will reveal the probable cause of these remarkable differences. Of the total number of Italians, Austro-Hungarians and Russians, who were admitted into this country since July 1st 1880, 75.3 per cent. and 77 per cent. respectively, arrived here from July 1, 1900 to June 30, 1914, while of the total number of Germans admitted since 1880, 60 per cent. arrived prior to June 30, 1890 and only 19.4 per cent. since July 1, 1900. The exact data regarding the Irish were not obtainable from the records from which the above figures were extracted, but it is very probable that the percentages are more or less similar to those of the Germans, assuming that the difference of age upon arrival does not differ materially between the two nationalities. This means that the three nationalities in New York State first mentioned are mostly composed of young adults in the most productive period of life, while the Germans and Irish are mostly people past childbearing age, or at least in the later stages of that period when the birth rate is much lower. It is notable that the Canadians and British have a lower birth rate in New York than in their respective countries of birth. This may be due to the greater similarity of their age constitution to that of the native class and to their greater inclination and ability to adopt the American standard of living.

Attention is directed to the fact that 73.1 per cent. of all births to foreign-born women were to Italian, Russian and Austro-Hungarian mothers and that these races accounted for nearly 27 per cent. of all the births occurring in the State outside of New York City, although they furnished less than 7 per cent. of the total population.

From a strictly scientific standpoint these figures are not thoroughly reliable. For all practical purposes, however, there seems to be no adequate reason why they should not be used. If anything the birth rates of the foreign-born are probably too low, since it is likely that the percentage of each foreign nationality to the total population was not as great in 1916 as it was in 1910, owing to the practical cessation of all immigration during the years of 1915 and 1916. This would probably more than offset any increase that may have occurred in the foreign population of the State from an influx of these people from other states, attracted by better industrial conditions, etc. It may therefore be assumed, that whatever error there may be, would, if corrected, but emphasize the point that the birth-rate of native women in New York State is undoubtedly as low, or lower, than that of France, and that the birth rate of the foreign-born woman is almost twice as great as that of the native woman.

The above birth rates (17.2 for native women and 43.8 for foreign women) are not fairly comparable owing to the great differences between the age constitution of each element. There is not only a greater proportion of married women from 15 to 45 years of age among the foreign class (according to the 1910 U. S. Census it amounted to 70 per cent. as against 52 per cent. for native women), but a larger percentage of them are between the ages of 21 and 30, the period of greatest productivity.

To illustrate the point Eastman presents the table, here given as Table VIII.

TABLE VIII.

A comparison of the crude, legitimate and illegitimate birth rates of native and foreign-born mothers. (From Eastman.)

	Birth rates.		
	Births to native-born mothers.	Births to foreign-born mothers.	Excess percentage of births to foreign-born mothers.
Number of births per 1,000 inhabitants	17.2	43.7	154
Number of legitimate births per 1,000 married women 15 to 44 years of age	137.1	253.2	85
Number of illegitimate births per 1,000 single, widowed or divorced women 15 to 44 years old................	2.1	3.2	52

To indicate the reliability of the New York figures Eastman compares them with Kuczynski's data for Massachusetts in 1895. The results are shown in Table IX.

TABLE IX.

A comparison of the birth rates in Massachusetts in 1895 and in New York in 1916. (From Eastman.)

	Massachusetts, 1895.	New York, 1916.
Births per 1,000 native population	17.03	17.2
Births per 1,000 foreign-born population	52.16	43.7
Births per native adult female population	* 48.78	† 48.6
Births per foreign-born females	* 107.29	† 104.2
Births per married native women of child-bearing age	* 142.47	† 137.1
Births per married foreign-born women	* 251.76	† 253.2

* Ages 14–50.
† Ages 15–44.

It is clear that the agreement is close. The same result has also been got by Hill (24).

Altogether there can be no doubt that in those parts of the country where a relatively large proportion of the population is foreign-born, the fertility of the foreign-born women is greatly in excess of that of the native-born. Almost, if not quite, the first biological result of Americanization is to reduce the fertility of the marriages. This is, of course, to be expected on all of the following grounds:

(*a*) The young immigrant woman is generally speaking a strong, vigorous, healthy animal—pioneering stock in fact. Even in these times it requires a person of more than the average gumption—both of body and of character—to pull up roots in the homeland and venture to a new world.

(*b*) The standard of living of the immigrant is relatively low, and this has virtually always and everywhere meant a high birth rate.

(*c*) The first broad economic result of Americanization is a raising of the standard of living. This makes itself felt in the first generation (native-born of foreign parents) in respect of the birth rate.

It would appear that this particular phase of the general problem of immigration, which causes some persons such great alarm, is one which in a generation or two adjusts itself quite automatically. Foreign-born women in this country do have a high birth rate, but their descendants very quickly slough off this attribute of barbarity, as it is regarded by some.

B. Illegitimacy.

While having no direct bearing upon our main problem, the question of illegitimate birth rates is an interesting one on its own account. It has been by many persons considered to be an index of morality, but most statisticians who have studied the question are inclined to question or deny entirely its significance in this regard. Eastman has shown (cf., Table VIII *supra*) that the illegitimate rate, *on the basis of single, widowed and divorced women*, is higher for the foreign than the native-born women in New York State. But it is also of interest to reckon the illegitimate rate on the basis of 1000 *total births*. The result of this method for the B. R. A. in 1919 is shown in Table X, which is modified from a table in Birth Statistics, 1919, pp. 16 and 17. Those states from which the returns are known to be incomplete are omitted, but their results are included in the figures for the Registration Area as a whole.

The illegitimate rate per 1000 total births is more than three times higher for native-born than for foreign-born mothers as a whole. For no one of the groups of foreign-born mothers does the illegitimate rate on this base approach in magnitude that for the native-born mothers. In the separate states the few exceptionally high rates for foreign-born mothers are based in every case upon fewer than 5 illegitimate births, in which case, of course, no reliance is to be placed upon the single rate by itself.

These facts disclose an interesting statistical paradox. Table VIII

TABLE X.

Number of illegitimate births (exclusive of stillbirths) per 1000 total births for white children, by country of birth of mother, 1919.

(Numbers are shown *in italics* where illegitimate births are less than 5.)

Area.	Country of birth of mother.													
	Total.	United States.	Foreign country.											
			Total.	Austria; includes Austrian Poland.	Hungary.	Canada.	Denmark, Norway and Sweden.	England, Scotland and Wales.	Ireland.	Germany; includes German Poland.	Italy.	Poland (not specified).	Russia; includes Russian Poland.	Other foreign countries.
Registration Area	12.0	14.9	4.1	3.9	5.1	10.0	5.5	8.6	8.7	5.6	1.5	4.1	2.1	3.7
Connecticut	9.1	15.0	4.4	6.3	*3.7*	8.9	11.2	*6.7*	4.7	—	2.5	7.5	3.1	*4.0*
District of Columbia	18.3	12.4	*2.6*	—	—	—	—	—	—	—	11.8	—	—	—
Indiana	11.6	12.3	2.3	3.7	*5.4*	—	—	*4.9*	—	*2.7*	—	*1.2*	—	—
Kansas	5.8	5.9	2.2	—	—	—	—	*9.3*	—	—	—	—	*2.3*	*3.8*
Kentucky	9.9	9.9	*2.3*	—	—	*47.6*	—	—	—	—	—	—	—	—
Maryland	18.6	20.9	3.0	*2.4*	—	—	*25.0*	—	—	*12.3*	—	*2.5*	*1.5*	*14.7*
Michigan	12.7	15.5	5.5	5.6	*2.4*	9.0	10.3	15.6	*11.0*	5.8	*1.6*	3.7	2.3	5.5
Minnesota	16.0	18.8	5.7	3.4	*3.7*	10.8	6.2	19.1	—	*3.3*	—	*7.5*	*4.5*	6.3
New York	9.3	14.7	3.8	3.8	6.2	20.1	4.9	12.0	9.1	5.6	1.2	5.6	1.8	6.0
North Carolina	14.8	14.1	—	—	—	—	—	—	—	—	—	—	—	—
Ohio	13.1	15.7	3.5	3.4	4.9	12.7	*5.5*	5.7	*4.0*	7.5	*1.1*	*1.1*	2.0	4.1
Oregon	13.6	14.5	5.8	—	—	*11.1*	—	*6.0*	—	*7.2*	*13.2*	—	*14.2*	*3.1*
Pennsylvania	16.3	22.1	4.6	4.1	6.4	*11.1*	10.4	10.0	12.7	9.1	1.9	5.6	3.3	5.3
South Carolina	16.9	16.9	—	—	—	—	—	—	—	—	—	—	—	—
Utah	7.8	8.4	3.7	—	—	—	*5.1*	*2.9*	—	*13.7*	—	—	—	*2.4*
Virginia	18.2	18.5	—	—	—	—	—	—	—	—	—	—	—	—
Washington	9.5	10.6	5.3	*6.7*	—	14.9	4.9	*4.3*	*7.6*	*3.8*	*3.1*	—	—	*1.5*
Wisconsin	11.8	13.4	3.9	3.2	*5.3*	*4.6*	*3.1*	*5.4*	—	6.1	*1.3*	5.3	*1.6*	*3.7*

shows that the illegitimate rate is higher per 1000 foreign-born women capable of having an illegitimate baby, than it is per 1000 native-born women in a similar social situation. But per 1000 total births the illegitimate rate is smaller for foreign- than for native-born mothers. The explanation is, of course, simple. There are so many more total births per 1000 foreign-born child-bearing women than per 1000 native-born that the illegitimate rate per 1000 births is bound to be low because the denominator of the rate fraction is large.

The essential point is that brought out in Table VIII. The foreign-born, unmarried, widowed or divorced woman is more likely to have a child than a similarly situated native-born woman. This probably does not mean better morals on the average in the latter case, but simply better judgment, which in turn means higher intelligence.

C. Stillbirths.

Data for stillbirths are not particularly accurate in the United States because of local differences in the legal definition of a stillbirth. Regarding this matter the Census Bureau (Birth Statistics, 1918, pp. 28 and 29) makes the following comments:

> Interpretation of the data relating to stillbirths must be made with extreme caution, as the term ‘‘ stillbirth ’’ is not used in the same sense in the various states and the percentage of the completeness of these reports is not known. The Model Law reads: A stillborn child shall be registered as a birth and also as a death, . . . provided that a certificate of birth and a certificate of death shall not be required for a child that has not advanced to the fifth month of uterogestation.
>
> Of the states in the birth registration area, Minnesota, New York, and North Carolina have similar provisions in their laws. The District of Columbia requires a certificate for a fetus ‘‘ passed the fifth month.’’ The Indiana law reads ‘‘ seven months’ gestation and over,’’ and the remaining states use the term stillbirth, but do not define it.
>
> In Maryland special efforts have been made to secure uniform registration of stillbirths of even the earlier months of uterogestation, so that the data for this state are not comparable with those for other states. At first thought these differences in the various state laws seem to limit greatly the comparability of the data, but the fact that the figures of those states requiring reports only of children which have reached the fifth month approximate the figures of those states which do not define the term ‘‘ stillbirth,’’ warrants the conclusion that in the latter states only stillbirths of the later months are for the most part reported, so that the figures are fairly comparable. Moreover, it is undoubtedly true that stillbirths of the later months of uterogestation are well reported, because such stillbirths are interred, and burial cannot legally take place without a burial permit, issued upon receipt of the physician’s certificate of death.

All data relating to stillbirths have been compiled from transcripts of stillbirths reported as births. Undoubtedly the total numbers of stillbirths would have been somewhat larger had data been compiled from transcripts of stillbirths reported as deaths, for foundling stillbirths would then have been included; but as most of the important facts relating to stillbirths are recorded on the birth certificates only, the Bureau of the Census gathered the information from this source.

For reasons, presumably connected with economy (most certainly of the false variety), all data on stillbirths are omitted in the 1919 Birth Statistics. We shall therefore have to take 1918 for the latest figures.

Table XI (from Birth Statistics, 1918, in rearranged form) gives the data as to stillbirths by country of birth of mother. To this table has been added a final column giving the average total fecundity of the several groups of mothers, as of the same year. The Registration Area is not quite the same for the figures of the last column and the next to the last, but the error so introduced is negligible for the use to which the figures are here put.

TABLE XI.

Births, stillbirths, stillbirths per 100 live births, and average number of children ever born, for white children by country of birth of mother, 1918.

Country of birth of mother.	Births.	Stillbirths.	Stillbirths per 100 live births.	Average number of children ever born.
United States	838,000	30,891	3.7	3.1
Total foreign	314,780	12,392	3.9	3.9
Canada	13,861	651	4.7	3.5
Ireland	14,038	620	4.4	3.3
England, Scotland and Wales	11,069	473	4.3	3.3
Italy	71,373	2,989	4.2	4.4
Russia (includes Russian Poland)	60,269	2,369	3.9	3.3
Austria (includes Austrian Poland)	62,111	2,384	3.8	3.9
Other foreign countries	22,355	835	3.7	3.6
Denmark, Norway and Sweden	11,624	413	3.6	3.6
Germany (includes German Poland)	15,414	559	3.6	4.6
Poland (not specified)	14,534	525	3.6	4.2
Hungary	18,132	574	3.2	3.8

The stillbirth rate is higher, on the basis of total live births, for foreign- than for American-born women in general. In the case of Scandinavian, German, Polish and Hungarian mothers the stillbirth

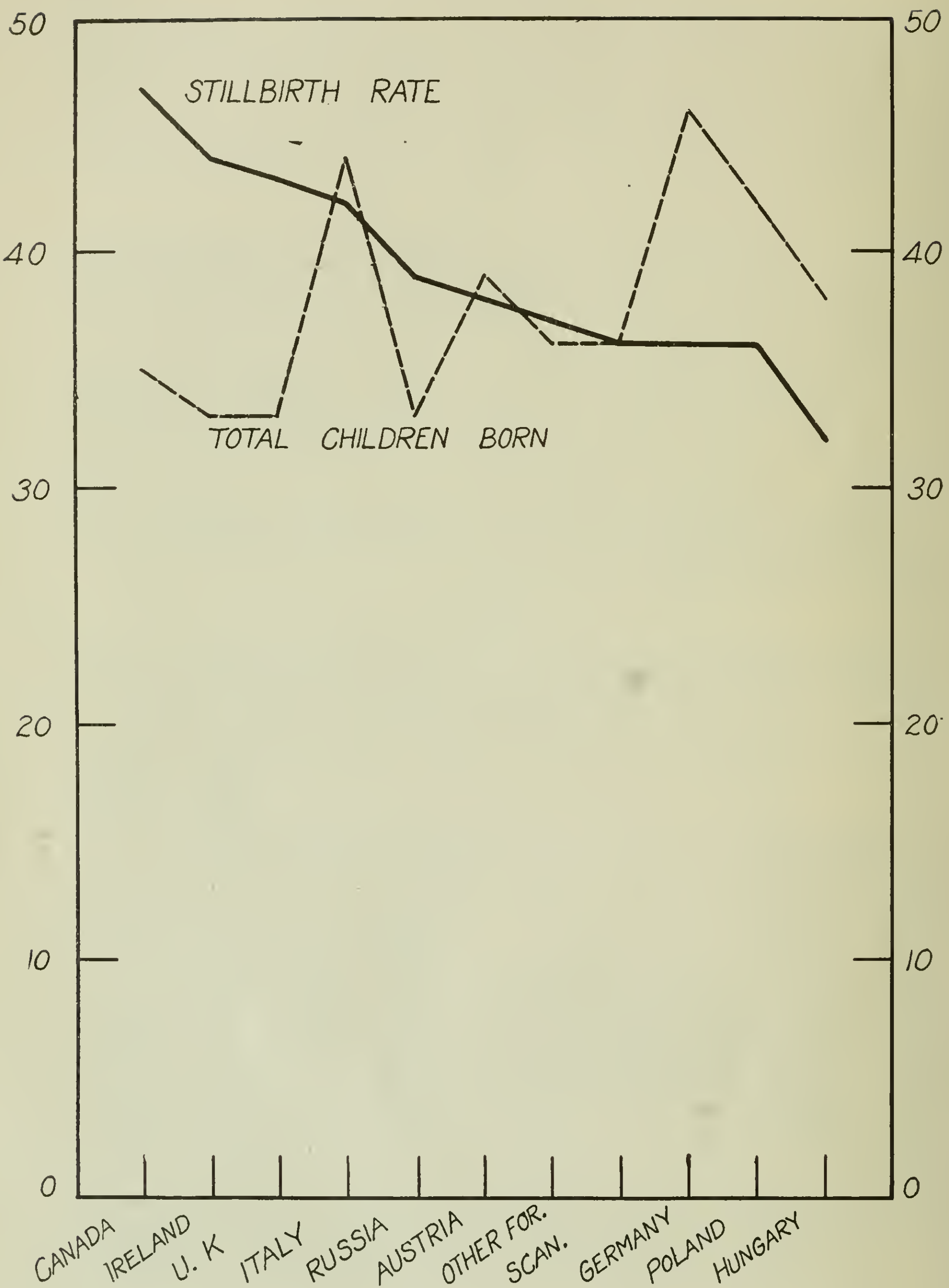

Fig. 11. Showing the relations between the stillbirth rate and the total number of children which have been born.

rate is lower than for the native-born mother. One's first thought is that the higher stillbirth rate of the other foreign-born mothers, as compared with the native-born, is due to the higher fertility of the former group. Comparing the fourth with the third column of Table XI gives little support, however, to such a view.

On the contrary, Fig. 11, which is plotted from the data of Table XI, seems rather definitely to indicate that the opposite relation holds on the whole. Namely, the more children a woman has had the less likely is the next birth to be a stillbirth.

V.

MORTALITY.

From published official data it is impossible to get any comprehensive or precise information as to the relative mortality of the different racial groups in this country. The fault is not so much with the original data contained in the death certificates, but with the fact that this material is not compiled, and by publication made available for the public information. The most that can be done is to learn something about the comparative mortality of the native- and foreign-born as total groups.

For the purpose of indicating what the facts in this restricted field show, Tables XII and XIII have been prepared. Table XII shows, for the six states, Connecticut, Massachusetts, Minnesota, New York, Pennsylvania and Wisconsin taken together, the population and deaths in 1910, divided according to whether the decedents were (*a*) native-born of native parents, (*b*) native-born but of foreign or mixed foreign and American parentage, and (*c*) foreign-born, and also by sex and age.

The six states chosen were taken because of their representative character in respect of different industries, and because of their large foreign-born populations. Together the states included in Table XII had in 1910 a total population of 25,205,393 individuals about each of whom age, sex, nativity, and parent nativity were definitely known. This is a respectable total number, and tends to give confidence in the results.

From the data of Table XII have been calculated death rates specific for age and sex for each of the three groups. These death rates are exhibited in Table XIII. Unless some arithmetical error has been committed in these calculations and overlooked, they represent

TABLE XII.

Population and deaths by sex, age and nativity, in six selected states, 1910.

Age.	Native-born males of native parentage.		Native-born females of native parentage.		Native-born males of foreign or mixed parentage.		Native-born females of foreign or mixed parentage.		Foreign-born males.		Foreign-born females.	
	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.
Under 1 year . .	133,312	17,414	129,877	13,284	151,852	24,298	147,861	18,403	1,678	202	1,546	190
Under 5	640,590	23,449	626,017	18,651	684,231	34,440	671,771	28,547	24,433	705	23,852	654
5–9	572,115	1,996	560,499	1,802	563,975	2,229	558,630	2,103	77,482	303	76,590	288
10–14	536,371	1,210	528,686	1,165	526,755	1,220	521,939	1,130	97,286	241	96,169	232
15–19	509,528	1,637	516,632	1,559	486,348	1,798	496,577	1,553	188,413	901	189,223	636
20–24	464,304	2,062	487,573	1,977	378,932	2,169	408,925	1,966	421,031	2,470	352,597	1,508
25–29	412,284	1,985	420,361	2,000	300,913	2,182	323,635	2,011	496,717	2,963	370,220	1,985
30–34	353,853	1,815	355,736	1,687	263,865	2,642	284,009	2,032	438,641	3,049	326,409	2,086
35–39	324,381	1,981	319,080	1,619	252,168	2,945	267,062	2,127	400,235	3,647	312,995	2,439
40–44	270,568	1,872	266,494	1,653	201,219	2,713	212,287	2,020	359,062	4,170	279,084	2,566
45–49	233,522	1,951	230,752	1,689	166,141	2,669	173,051	1,966	303,204	4,618	242,098	2,872
50–54	217,332	2,558	214,152	2,213	135,964	2,652	137,891	2,001	234,597	4,651	192,357	3,180
55–59	172,234	3,018	170,721	2,512	75,189	2,027	76,818	1,593	163,464	4,687	147,355	3,577
60–64	141,568	3,664	149,246	3,007	39,264	1,411	42,071	1,209	139,908	5,631	136,559	4,918
65–74	186,961	8,438	207,898	8,240	33,910	1,913	38,058	1,952	175,063	11,388	178,827	11,079
75–84	68,992	6,785	85,992	7,749	7,346	905	9,144	1,001	61,395	7,944	67,783	8,478
85–94	10,448	1,955	15,356	2,765	1,045	271	1,431	397	8,883	2,298	10,845	2,586
95 and over . .	285	92	591	181	35	13	48	13	349	156	498	208
Totals	5,115,336	66,468	5,155,786	60,469	4,117,300	64,199	4,223,347	53,648	3,590,163	59,822	3,003,461	49,292

a very close approximation to the true forces of mortality, operating in each defined group. The reader should not confuse them with crude death rates. They are true specific death rates, the specificity extending to the three variables age, sex and nativity with parent nativity.

TABLE XIII.

Specific death rates per 1,000 living of same sex and age, from Table XII.

Age.	Native white of native parentage.		Native of foreign or mixed parentage.		Foreign born white.	
	Male.	Female.	Male.	Female.	Male.	Female.
Under 1 year........	130.63	102.28	160.01	124.46	120.38	122.90
Under 5.............	36.61	29.79	50.33	42.53	28.85	27.42
5–9...........	3.49	3.21	3.95	3.77	3.91	3.76
10–14..........	2.26	2.20	2.32	2.17	2.48	2.41
15–19..........	3.21	3.02	3.70	3.13	4.78	3.36
20–24..........	4.44	4.05	5.72	4.81	5.87	4.28
25–29..........	4.81	4.76	7.25	6.21	5.97	5.36
30–34..........	5.13	4.74	10.01	7.15	6.95	6.39
35–39..........	6.11	5.07	11.68	7.96	9.11	7.79
40–44..........	6.92	6.20	13.48	9.51	11.61	9.19
45–49..........	8.35	7.32	16.07	11.36	15.23	11.86
50–54..........	11.77	10.33	19.51	14.51	19.83	16.53
55–59..........	17.52	14.71	26.96	20.74	28.67	24.27
60–64..........	25.88	20.15	35.94	28.74	40.25	36.01
65–74..........	45.13	39.63	56.41	51.29	65.05	61.95
75–84..........	98.35	90.11	123.20	109.47	129.39	125.08
85–94..........	187.12	180.06	259.33	277.43	258.70	238.45
95 and over.....	322.81	306.26	371.43	270.83	446.99	417.67

The data of Table XIII are shown graphically in Figs. 12 and 13.

The first point which strikes one from the examination of these specific death rates is that the infant mortality (death rate under 1) is *lower* for the foreign-born, in the case of both males and females, than for either of the other two classes. This result is a spurious one, arising from the following circumstances. To get entered in the deaths under 1, in this country, a foreign-born baby must have first been born abroad, survived the trip to this country and then died here. Now in view of the fact that the heaviest incidence of mortality in the first year of life is known to be always and everywhere in the first month of the first year, it necessarily follows that some unknown, but fairly large proportion of the new-born babies of families about to immigrate to the United States in 1910 died, either before the family left the old country, or while they were upon the ocean. Consequently they would never appear in our statistics. But there would be left to die in this country during the first year of life a smaller number of foreign-born babies, and furthermore a selected kind of babies, namely the survivors of the period of heaviest incidence of infant mortality. So we must conclude that the foreign-born mortality under 1 is not accurately comparable with the native-born mortality in the same age group. The influence of this spurious element makes itself felt throughout the "Under 5" group.

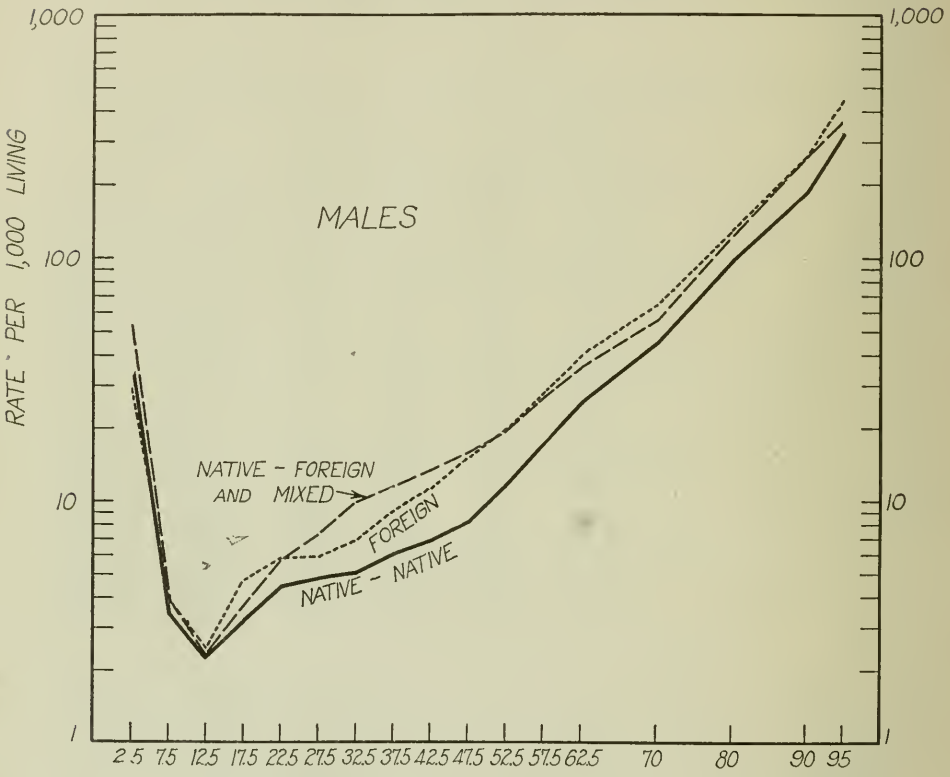

FIG. 12. Specific death rates for (*a*) native-born males of native parentage (solid line), (*b*) native-born males of foreign or mixed parentage (dash line), and (*c*) foreign-born males (dot line) in six selected states (see text) in 1910.

After this period is passed the specific rates are *lower,* at all ages right away to the end of life, for the native-born of native parents than for either of the other two groups, in both males and females, with the single exception that the native-born of foreign or mixed parentage rate for females in the age group 95 and over is a little lower than the rate for native-born women of native parentage. No significance is to be attached to this slight deviation from the rule at the end of the curve, because all rates for that age group are subject to large probable errors because of the meager numbers involved.

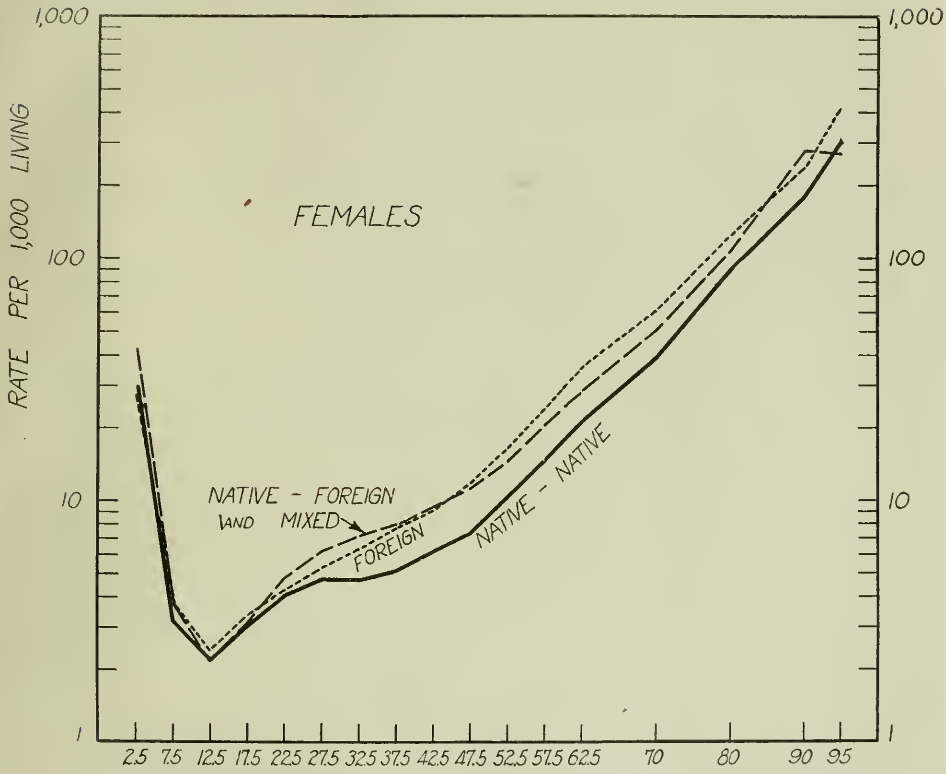

FIG. 13. Specific death rates for (*a*) native-born females of native parentage, (*b*) native-born females of foreign or mixed parentage, and (*c*) foreign-born females in six selected states in 1910. Significance of lines as in Fig. 11.

The superior estate of the native-born of native parents in respect of mortality undoubtedly arises from the combined action of the following factors: (*a*) *superior intelligence*, not perhaps in an absolute or abstract sense, but concretely in respect of awareness of and ability to grapple with the particular kind of environmental difficulties connected with living in the United States. This is probably the most important single factor. The native-born American of native parentage has a pretty fair fund of knowledge and tradition as to how best to contend with strictly American dangers—hygienic, economic and other.

(*b*) *Superior physical environment,* associated with a higher general standard of living. If one were obliged to put into a single statement the most general and important reason why genuine immigrants (meaning those intending to stay) now come to our shores, it would run, I think, in about this form: that above everything else the immigrant desires and intends to raise the standard of living of his family and descendants. After two generations have been born in this country the result has either been achieved or it has not. If it has not, the family that started the enterprise has, generally speaking, either died out or gone back home. There can be no question about the fact that, by and large, and in most parts of the country, the standard of living of the native-born of native parentage is definitely higher than that of either of the other two groups.

On the other hand, the relations of the specific mortality curves are, for the native-born of foreign or mixed parentage, most curious, and not entirely easy to explain satisfactorily. Taking the male data first, we see that in youth (from age 10 to about age 20) the specific rates are higher for the foreign-born. The curves cross at about age 22.5, and all through young adult and early middle life—up to age 52.5—the specific rates for the foreign-born are lower than those of the native-born of foreign or mixed parentage. From then on to the end of the life span the specific rates for the foreign-born are again higher than those for the native-born of foreign or mixed parentage.

The pertinent factors in the case appear to be as follows:

1. During childhood the foreign-born individuals probably receive on the whole less effectively good care in a hygienic sense than native-born children of parents of foreign or mixed parentage, on the ground that the recent immigrants understand our environment less well. Furthermore there is an extra occupational hazard upon foreign-born lives even in the childhood ages. So then the higher specific rates of childhood on the foreign-born are exactly what we should expect.

2. During early adult and middle life we have, apparently:

	Foreign-born.	Native-born of foreign or mixed parents.
a. Physical environment and standard of living	Worse	Better
b. Occupational hazard	Worse	Better
c. Innate biological constitution	Better	Worse
d. Racial constitution of the population	Better	Worse

Rubrics *a* and *b* need no explanation or argument, so far as I can see. Rubric *c* is meant to express the fact that immigration, even here and now, is a real form of pioneering. Only the relatively superior—in comparison with the group *out of which they come*—in physique, courage and general mental soundness have the initiative and strength of character to undertake the big adventure of starting anew in a strange country. That this selective action has its effect upon mortality, and that this effect is a considerable one, admits of no doubt (9).

Under rubric *d* is included whatever differences in race stock there may be between the foreign-born and the native-born of foreign or mixed parentage. Dublin and Baker (10) consider, on the basis of a study of New York and Pennsylvania alone, that the difference in the death rates that we are now discussing is practically entirely due to the "predominance of the Irish, German and British stocks among the first generations of Americans" in the age period 25–44. That this is a factor admits of no doubt. And it is an innate, biological one in last analysis. It does not seem to me, however, that Dublin and Baker have entirely demonstrated that it is the only, or even overwhelmingly important factor in the case.

In the study of the mortality of different race stocks in this country lies a wonderful opportunity for a piece of analytical work, the results of which could not fail, if it were carefully and sagaciously done, to throw a flood of light on some of our most pressing social as well as public health problems. What is wanted here is quantitative fact, not opinion. And it can be got, if only somebody sufficiently interested and public-spirited will finance the business.

So far we have been focussing attention upon the male curves. If we now turn to the female data, however, it is immediately apparent that whatever differences appear are essentially of degree not kind. In their general course the three female curves are essentially like the three male curves.

Altogether it may fairly be said that the exhibition of specific death rates, meager as is the specificity in respect of race, has served to define a large and important problem. If we could get compilations of deaths and populations separately for each racial group by age, sex, occupation, nativity and parent nativity, how much farther we could carry our knowledge of the factors influencing human mortality.

Already the attempt has been made to carry the analysis of racial factors in mortality in this country farther. Dublin and Baker (*loc. cit.*), by means of data specially obtained from the Census Bureau, have recently studied in a most interesting and profitable manner the mortality of different race stocks in New York and Pennsylvania. Dublin (11) earlier had made a similar study for certain race stocks in New York.

Dublin and Baker examined separately certain important causes of death. Their findings were as follows:

> *Tuberculosis of Lungs.*—The outstanding feature relative to pulmonary tuberculosis is the very unfavorable position of the Irish. Both sexes suffer extraordinarily from this disease during the greater part of life (25–84). At 25–44 the rates for Irish males are twice as high as for natives, and in the age period 45–64 the excess is even greater. The death rate among Irish males from tuberculosis continues high even at the advanced ages. The facts with regard to the Irish in the two states studied, are very different indeed from those in the native country, both as to the high rates obtained and the general configuration of the mortality curve. German and British males also show higher tuberculosis rates than the native-born at the older age. On the other hand, the Italians, Russians and Austro-Hungarians present comparatively low tuberculosis death rates. At most of the age periods they are even lower than for the native-born. Italian females in New York show a striking exception to the usual rule in that their rates are higher than those for males, especially in early life. Otherwise there are no important differences in the several race groups with reference to this disease.
>
> *Cancer.*—Practically every foreign race group shows higher cancer rates than do the native-born. Italian males and females offer the only clear-cut exceptions. At the age period 45–64, foreign-born males show rates from 50 to 100 per cent. higher. The conditions among the Irish are strikingly bad. Among females at ages 45–64, the Germans and the British also show higher rates than do the native-born, but the excess is not as marked as it is among the Irish.
>
> *Organic Diseases of the Heart.*—Again the Irish present the highest rates, followed by the British and the Germans. The conditions prevailing among these from Austria-Hungary, Russia and Italy are, on the whole, very favorable with regard to this disease. It is very difficult to understand the reason for the aberrant death rates among the Irish in Pennsylvania and New York, for they are so much higher than are those for the native stock and for the Irish in their own country.
>
> *Pneumonia, all forms.*—From ages 45–84, the rates of the native-born are much the lowest. The Irish are again under the greatest handicap, their rates being about three times as high at ages 45–64. The rates for Italians are very unfavorable also at ages 45–84. The disadvantage of the Germans, British and Russians is less among females than among males. The position of the Austro-Hungarians is very much the same as the native-born at 25–64.
>
> *Bright's Disease.*—Again, we must record the very unfavorable position which the Irish occupy. From 25–84 years of age, the rates for this nationality are about 100 per cent. higher than for the native-born; among females, the excess is even greater. At ages 45–84, the rates from Bright's disease are high for Austro-Hungarians, Russians, Germans and British. The position of the Italians is quite favorable.

Violence (excluding suicide).—Deaths from this cause are largely from accident, and we find, of course, that the positions of the several race groups are determined by the extent to which they engage in occupations that expose them to such dangers. The figures are especially striking for Pennsylvania where large numbers of immigrants find employment in mills and mines. Males of all the important foreign groups show very high rates from this cause, the worst conditions prevailing among the Austro-Hungarians, followed by the Irish and Italians.

Their whole study Dublin and Baker summarize as follows:

1. Of the three main groups of the white population in Pennsylvania and in New York, (*a*) native-born of native parents, (*b*) native-born of foreign or mixed parentage, and (*c*) foreign-born, the first has the lowest mortality. This is true for both sexes and for virtually every age period, but it is most marked at the adult ages.

2. The foreign-born and the native-born of foreign or mixed parentage, agree much more closely with each other than with the native stock. An interesting exception presents itself, however, at ages 25–44, at which period the foreign-born have a great advantage over the native-born of foreign or mixed parentage. The reason for this is the predominance of the Irish, German and British stocks among the first generation Americans at this age period. After age 45, these two groups of the foreign stock are of the same racial extraction, and their death rates are in very close agreement.

3. The death rates of the component groups among the foreign-born vary considerably. The Austro-Hungarians, Russians, and Italians present altogether favorable conditions, while the British, Germans and Irish show death rates very greatly in excess. This is especially true of the Irish, whose mortality is about double that of the native stock. The rates for the Germans, British and Irish are much higher in America than in their own countries. Pulmonary tuberculosis, pneumonia and degenerative diseases including heart disease, Bright's disease, and cancer are largely responsible for this unfavorable mortality.

4. The findings of the previous study for New York State are confirmed. The unfavorable conditions of life and work among foreign races to which attention was directed in the study for New York are found to prevail in Pennsylvania as well. The facts emphasize the necessity for special public health work for the people of foreign origin. The much more favorable economic conditions under which they live in the United States than in their own countries should result in lower death rates. But in several instances, we find that this does not prevail; the facts indicate on the whole, deterioration rather than improvement. Is it possible that our immigrants are not representative of the best in their native countries? It has often been supposed that the immigrants comprised the most vigorous among their own people; the results, however, do not confirm this impression, but suggest many questions for further inquiry.

5. It is very important that a study similar to this one be carried out as soon as the results of the 1920 census are available, to determine whether any differences of importance have appeared in the interval of ten years.

Interesting and valuable as is the study of Dublin and Baker it is

TABLE XIV.

Infant mortality from principal causes and by the nativity of the mothers—1916. (From Eastman.)

	Total births.	Deaths under one year from all causes.	Inf. Mort.	Deaths from Principal Causes.									
				Communicable diseases (1–14 inc.).		Respiratory Diseases (28–29 and 86–98 inc.).		Gastro-intestinal diseases (103–104 inc.).		Premature birth, congenital debility and malformations (150–153 inc.).		All other causes.	
				D's.	Inf. Mort.	Deaths.	Inf. Mort.	Deaths.	Inf. Mort.	Deaths.	Inf. Mort.	Deaths.	Inf. Mort
Total mothers	103,530	9,912	95.7	471	4.5	1,530	14.8	2,265	21.9	4,435	42.8	1,211	11.7
Total white mothers	102,834	9,760	94.9	463	4.5	1,505	14.6	2,239	21.8	4,372	42.5	1,181	11.5
Total colored mothers	696	152	218.4	8	11.5	25	35.9	26	37.4	63	90.5	30	43.1
Native white mothers	64,889	5,648	87.0	229	3.5	684	10.5	1,096	16.9	2,943	45.3	696	10.7
Total foreign white mothers	37,914	4,112	108.4	234	6.1	821	21.6	1,143	30.1	1,429	37.7	485	12.8
Italian mothers	12,998	1,259	96.9	90	6.9	313	24.1	317	24.4	402	31.0	137	10.5
Russian mothers (excluding Russian Poland)	3,665	377	102.9	18	4.9	64	17.5	128	34.9	126	34.4	41	11.2
Austro-Hungarian mothers (excluding Austrian Poland)	6,345	822	129.6	41	6.4	165	26.0	273	43.0	268	42.3	75	11.8
Polish mothers (including German, Austrian and Russian Poland)	4,703	577	122.7	37	7.9	116	24.7	191	40.6	171	36.3	62	13.2
Total for group	27,711	3,035	109.5	186	6.7	658	23.7	909	32.8	967	35.0	315	11.4
British mothers	1,869	134	71.7	6	3.2	14	7.5	22	11.7	68	36.4	24	12.8
Irish mothers	1,879	182	96.9	8	4.2	19	10.1	40	21.3	82	43.7	33	17.6
German mothers	2,296	283	123.3	7	3.0	48	20.9	80	34.8	117	51.0	31	13.5
Canadian mothers	2,219	243	109.5	13	5.9	43	19.4	45	20.2	107	48.2	35	15.8
Other foreign-born mothers	1,940	235	121.1	14	7.2	39	20.1	47	24.2	88	45.4	47	24.2
Total for group	10,203	1,077	105.6	48	4.7	163	16.0	234	22.9	462	45.3	170	16.7

TABLE XV.

Principal causes of deaths of infants under 1 year of age showing percentages due to certain elements of the population. (From Eastman.)

	Total births.	Births per cent.	Total deaths under one year.		Causes of death.									
					Communicable diseases (1–14 inc.).		Respiratory diseases (28–29 and 86–98 inc.).		Gastro-intestinal diseases (103–104 inc.).		Prenatal causes, etc. (150–153 inc.).		All other causes.	
			Deaths.	Per cent.	D's.	Per cent.	Deaths.	Per cent.	Deaths.	Per cent.	Deaths.	Per cent.	Deaths.	Per cent.
Total white mothers	102,834	100.0	9,760	100.0	463	100.0	1,505	100.0	2,239	100.0	4,372	100.0	1,181	100.0
Native white mothers	64,889	63.1	5,648	57.9	229	49.5	684	45.4	1,096	49.0	2,943	67.3	696	58.9
Total foreign-born mothers	37,914	36.9	4,112	42.1	234	50.5	821	54.6	1,143	51.0	1,429	32.7	485	41.1
Italian, Russian, Austrian and Polish mothers	27,711	27.0	3,035	31.1	186	40.2	658	43.7	909	40.6	967	22.1	315	26.7
Other foreign-born mothers	10,203	9.9	1,077	11.0	48	10.3	163	10.8	234	10.4	462	10.6	170	14.4

plain that a great deal further remains to be done along similar lines.

A very interesting study has been made by Eastman (12) of the relation of parental nativity to the infant mortality of New York State. It brings out in such a clear manner the significance of what are perhaps the two most important elements in determining rates of mortality, namely, innate biological constitution, on one hand, and general intelligence, on the other hand, that it will be of value to examine the results in some detail. Tables XIV and XV (which are Tables V and VI of Eastman's paper) show the data.

Commenting on Table XIV Eastman says:

> The striking dissimilarity existing in the relative importance of the several main groups of causes, as distinguished between the native- and foreign-born mothers, is at once apparent even on a casual review. The infant mortality from communicable diseases was almost 75 per cent. greater among children of foreign mothers than among the babies of native mothers; from respiratory diseases it was over 100 per cent. greater, and from gastro-intestinal diseases the excess was about 78 per cent.; but the rate from prenatal and other causes peculiar to early childhood was higher among the native element by over 20 per cent. Pursuing the analysis still further, by dividing the total number of foreign-born mothers into the same two groups adopted in the preceding tables, we find these differences much accentuated when the native stock is compared with the group comprising the Italians, Russians, etc. The other foreign-born mothers, considered as a class, also exceeded the native mothers in the death rates from communicable, respiratory, and gastro-intestinal diseases, but the rates from prenatal, etc., causes were identical.
>
> Although the table gives us a very fair idea as to the relative prevalence of the several causes among certain classes of the population, it fails to show the percentage of the complete number of deaths from any particular cause that was attributable to each of the three classes of the population.

This relation referred to is brought out in Table XV. Regarding this Eastman says:

> Considering first the deaths of children of native white mothers, it will be noted that although they formed 57.9 per cent. of all the deaths from all causes, they nevertheless contributed only 49.5 per cent. of the total deaths from communicable diseases, 45.4 per cent. of all the deaths from respiratory diseases, and 49 per cent. of the total mortality from gastro-intestinal diseases. At the same time, 67.3 per cent. of all the deaths from premature birth, congenital debility, congenital malformations, accidents at birth, etc., were of children of these mothers. In contrast with these it is interesting to observe that, notwithstanding the fact that only 42.1 per cent. of all the white infant deaths occurred to children of foreign-born mothers, 50.5 per cent., 54.6 per cent. and 51 per cent. respectively, of the complete infant mortality from communicable, respiratory and gastro-intestinal diseases were referable to them. As to the mortality from

prenatal causes, however, it is to be remarked that only 32.7 per cent. of the total from these causes occurred among this element in the population This table shows also that the high death rates from the first three groups of diseases, incident to the children of the foreign-born, were particularly attributable to the Italians, Russians, Poles and Austro-Hungarians.

We may therefore consider that our previous surmise has been corroborated, namely, that the chief causes of infant mortality among the native population originate for the most part in adverse prenatal conditions, but that among the foreign element, and especially the Italians, Poles, etc., the most frequent causes of death are communicable, respiratory and gastro-intestinal diseases.

The explanation of these results is undoubtedly in general terms as follows: The fewer deaths from prematurity and congenital defects among the children of Italian and Slavic mothers indicate that superiority of innate biological constitution which is generally associated with emigrating stocks. On the other hand, the high death rate among these infants from causes which Eastman classes as communicable, respiratory and gastro-intestinal is chiefly due to the mothers' ignorance. As Eastman says:

> It is not to be wondered at that they are the dominant causes among the foreign-born population, the majority of whom are poor, illiterate, without knowledge of English and almost wholly ignorant of the elements of modern sanitation, and inhabit, as a rule, the most congested districts of the large manufacturing centers.

Guilfoy (13) has also made an interesting study of the influence of race upon mortality in New York City. His results are in good accord with those of the other investigators which have been cited.

VI.

Vital Index.

The writer (6) has elsewhere suggested the term "vital index" to designate that measure of a population's condition which is given by the ratio of births to deaths within a given time. It may fairly be said that there is no other statistical constant which furnishes so adequate a picture as this of the net biological status of a population as a whole at any given moment. If the ratio 100 Births/Deaths is greater than 100 the population is in a growing and in so far healthy condition. If it is less than 100 the population is *biologically* unhealthy. Depopulation may not be actually occurring, if there is a sufficient amount of immigration to make up the deficiency in births. But fundamentally and innately the condition is not a sound one from a biological standpoint, though under certain circumstances it may be from a social standpoint. It is curious, in view of the obvious significance of this constant, the vital index of a population, that so little attention is paid to it by demographers. After much study of it (mostly unpublished) I am convinced that no single figure gives so sensitive a measure of the vitality of a nation, or any subgroup of people as this does. There appears to have been no adequate general discussion of it since that of Wernicke (14) in 1889, and even he does not use it in the most effective manner or form. Sundbärg (17) proposed its use as a "measure of civilization" of different peoples. Rubin (18) criticized Sundbärg, but only in respect of technique, proposing as a measure of civilization D^2/B in place of D/B, where $D =$ deaths and $B =$ births. During the present year Pell (25) has dealt with the idea implicit in the birth/death ratio, but in a most inadequate manner.

For the purposes and viewpoint of our present discussion this constant is peculiarly fitted. One of our main problems is to see what is to be the probable future course of the several racial elements in our population. No better indicator of this could be had than the vital indices of the different groups. Unfortunately we find ourselves restricted, just as in other parts of the discussion, by a lack of the raw data needful to undertake a properly detailed analysis. It will be necessary, as in all of the previous discussion, to make the best of a somewhat bad case.

In Table XVI are shown four vital indices for urban, rural and total births and deaths of each state in the B. R. A. for the years 1915 to 1918 inclusive.

The significance of the several indices is as follows:

$$\text{Vital Index } A = \frac{100 \text{ (Births of whites of native parents)}}{\text{Deaths of all native whites}}$$

In this index the births and deaths come from an identical group of the population. The children born were of course native, and their parents were also native-born. The deaths were of native-born, i.e., the same group as the parents of the births. All racial elements (white) are included in births and deaths, but all are Americans in the sense of nativity.

$$\text{Vital Index } B = \frac{100 \text{ (Births of whites, both parents foreign)}}{\text{Deaths of foreign-born whites}}$$

Here again both births and deaths come from an identical group.

The births are children of foreigners in this country. The deaths are of foreigners in this country.

$$\text{Vital Index } C = \frac{100 \text{ (Births of negroes)}}{\text{Deaths of negroes}}$$

This needs no discussion.

$$\text{Vital Index } D = \frac{100 \text{ (Births of whites)}}{\text{Deaths of whites}}$$

This is for comparison with *C*. Both *C* and *D* are true vital indices in the sense that the parents of the births in the numerator are drawn from the same population group as the deaths in the denominator.

Unfortunately on the basis of present published official compilations of statistics these four are the only significant vital indices which can be drawn up. For any really deep understanding of what the biological effect is of racial fusion, and of a new environment, on the net vitality of populations we ought to have a whole series of racially specific vital indices. Here again there is no practical hope of getting these from purely official sources. Some one must come forward and finance a comprehensive and thorough investigation along these lines from outside.

The facts about Indices *A*, *B*, *C*, and *D* are set forth in Table XVI. In this table a figure in *italics* indicates that the absolute number of births and deaths on which the index is based is in each case less than 100. It will be noted that there are few such cases, and that they are practically all among the negroes of the northern states.

This table presents many novel points of great interest. We may first compare vital indices *A* and *B*, which indicate the relative biological vigor of the native-born and the foreign-born populations in this country. Taking totals first we note that for each grouping and each year Index *B* is much larger than Index *A*. Except for the rural population *B* is more than twice as large as *A*. Generally speaking the foreign population produces in this country approximately two babies for every death. The native population (as defined in Vital Index *A*) produces only a small fraction over one baby for each death. In other words the native population, even when so broadly defined as by Index *A*, is in about the same state as France before the war, and not in as vigorous a state as the French population is now.

An examination of the same point by states is of interest. In Connecticut, in all but the influenza year 1918, Index *B* was approximately four times as large as Index *A*, and in 1918 it was three times.

TABLE XVI.

Vital indices of various elements in the population of registration states, cities in registration states, and rural portions of the registration states in the Birth Registration Area. (1915–18 inclusive.)

State and group.		1915—Vital Index.				1916—Vital Index				1917—Vital Index.				1918—Vital Index.			
		A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Connecticut	Cities	82.9	355.8	94.8	195.4	81.8	340.6	85.1	189.3	90.4	331.0	82.0	196.1	72.9	219.2	75.1	143.7
	Rural	75.7	292.0	*60.4*	149.0	73.5	282.9	*81.3*	146.4	77.8	293.2	*82.7*	149.2	65.7	212.2	65.0	120.2
	Total	80.5	339.7	86.0	180.9	79.2	326.6	84.3	176.7	86.4	322.6	82.1	182.8	70.8	217.7	73.4	137.3
District of Columbia	Total	117.0	97.5	85.2	123.0	119.7	93.5	87.3	125.9	126.6	103.7	85.5	131.7	102.1	78.5	64.7	104.2
Indiana	Cities	*	*	*	*	*	*	*	*	144.0	174.5	71.5	158.2	124.1	142.5	66.3	134.9
	Rural	*	*	*	*	*	*	*	*	172.5	54.3	59.1	166.3	153.2	53.1	53.8	149.0
	Total	*	*	*	*	*	*	*	*	162.7	121.8	68.2	163.3	142.8	108.5	63.3	143.6
Kansas	Cities	*	*	*	*	*	*	*	*	149.8	103.6	70.6	150.0	116.2	72.3	65.6	114.7
	Rural	*	*	*	*	*	*	*	*	223.7	58.1	74.5	208.2	190.4	50.5	67.4	177.9
	Total	*	*	*	*	*	*	*	*	207.3	68.6	72.2	195.3	171.9	50.1	66.4	162.2
Kentucky	Cities	*	*	*	*	*	*	*	*	135.8	26.6	47.4	123.1	105.6	25.1	34.7	98.5
	Rural	*	*	*	*	*	*	*	*	241.4	35.9	91.0	236.4	203.1	38.6	74.5	199.6
	Total	*	*	*	*	*	*	*	*	221.9	29.7	76.0	202.9	183.1	29.3	60.3	177.0
Maine	Cities	75.6	188.6	*71.4*	131.5	73.8	163.8	*25.0*	122.4	79.4	173.8	*75.0*	128.4	70.7	124.9	*53.8*	106.2
	Rural	105.6	156.0	*87.5*	136.4	105.7	146.2	*18.7*	135.9	115.0	161.6	*8.3*	145.5	96.5	112.5	*21.1*	119.9
	Total	98.8	169.0	*80.0*	135.1	98.1	151.3	*21.9*	132.3	106.3	166.7	*35.0*	143.0	90.2	117.8	*34.4*	116.2
Maryland	Cities	*	*	*	*	138.3	166.4	82.9	136.8	137.7	152.1	80.8	152.7	96.2	107.7	63.7	105.9
	Rural	*	*	*	*	177.0	90.1	128.6	173.5	173.3	82.6	125.1	168.7	129.8	67.6	92.8	126.3
	Total	*	*	*	*	157.5	144.9	106.8	164.6	155.1	132.2	103.1	160.1	111.9	96.8	78.8	114.9
Massachusetts	Cities	86.7	276.2	113.2	186.4	87.0	251.2	101.1	176.3	92.1	246.5	111.3	179.7	70.1	171.5	97.0	129.6
	Rural	80.5	226.4	70.6	145.1	79.0	202.1	73.5	135.3	77.4	207.4	128.4	135.6	63.0	147.8	86.2	104.8
	Total	85.1	267.1	105.8	177.0	85.1	242.3	96.0	167.2	88.5	239.6	113.3	169.9	68.5	167.4	95.4	124.5
Michigan	Cities	104.5	234.5	86.3	205.4	138.0	227.2	66.7	195.7	143.0	226.4	79.1	198.5	132.3	192.6	86.8	179.2
	Rural	182.2	143.5	83.1	197.9	172.2	140.5	68.1	186.7	171.7	139.4	64.1	185.3	155.9	123.8	77.0	167.4
	Total	165.0	187.8	85.2	201.2	157.2	184.4	67.1	190.9	158.8	184.2	74.5	191.5	145.2	159.7	84.1	173.0
Minnesota	Cities	173.0	166.3	59.9	211.5	163.2	148.2	51.4	194.5	170.8	136.3	67.7	194.5	137.0	105.1	61.1	156.4
	Rural	282.0	118.1	*18.1*	264.2	277.3	104.6	*88.2*	252.8	289.9	96.7	*50.0*	254.9	208.0	79.3	*42.9*	195.1
	Total	240.7	134.9	51.8	244.9	232.1	120.2	55.3	230.5	242.1	111.2	66.1	231.5	181.3	88.7	59.2	180.7
New Hampshire	Cities	70.3	293.7	*150.0*	163.0	72.4	248.4	*200.0*	153.9	69.2	239.5	*114.3*	147.1	60.9	164.1	*100.0*	113.7
	Rural	90.9	172.0	*50.0*	124.7	90.0	150.3	*30.0*	121.3	87.4	133.3	*60.0*	113.3	69.7	101.0	*20.0*	89.3
	Total	82.8	240.9	*110.0*	140.9	83.1	206.7	*69.2*	135.4	79.8	195.2	*91.7*	128.4	65.9	139.3	*71.4*	100.7
New York	Cities	88.4	273.5	94.9	179.5	88.5	255.4	101.6	172.5	95.6	246.0	96.3	175.2	79.4	187.1	84.5	137.1
	Rural	109.6	140.7	85.3	128.1	107.4	138.5	79.7	125.6	105.1	128.8	69.9	121.4	88.5	106.6	54.8	101.2
	Total	95.8	253.6	93.6	166.5	94.2	238.2	98.5	160.8	98.5	228.7	92.6	161.8	82.1	176.3	80.8	128.4

State and group.		1915—Vital Index.				1916—Vital Index.				1917—Vital Index.				1918—Vital Index.			
		A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
North Carolina	Cities	*	*	*	*	*	*	*	*	148.0	*60.9*	78.1	147.5	*99.1*	32.9	62.2	98.7
	Rural	*	*	*	*	*	*	*	*	266.8	*160.3*	190.3	270.4	223.7	47.0	145.0	224.4
	Total	*	*	*	*	*	*	*	*	255.4	106.3	173.1	258.2	209.2	41.6	33.8	209.3
Ohio	Cities	*	*	*	*	*	*	*	*	136.1	210.5	64.6	167.1	117.2	160.2	66.1	39.3
	Rural	*	*	*	*	*	*	*	*	157.5	113.3	73.8	156.3	138.2	98.5	70.1	137.5
	Total	*	*	*	*	*	*	*	*	147.0	182.2	67.2	162.0	127.7	143.3	67.2	138.5
Pennsylvania	Cities	117.2	273.3	95.2	179.2	110.4	253.8	87.6	166.5	114.6	243.1	74.4	166.3	81.1	153.7	59.3	112.5
	Rural	152.5	385.7	87.4	207.6	141.8	353.0	80.7	191.0	146.7	332.5	76.0	192.9	104.0	174.2	56.4	128.3
	Total	135.7	314.9	93.3	193.1	126.7	290.4	86.0	178.4	130.9	276.3	74.7	179.0	92.6	162.2	58.6	120.0
Rhode Island	Cities	74.3	219.5	83.2	158.3	72.5	216.4	76.1	152.7	79.2	217.8	89.5	159.2	64.0	172.5	91.6	126.0
	Rural	70.5	319.4	*65.8*	157.4	79.7	331.9	*113.6*	175.2	84.0	358.7	*123.8*	182.9	63.8	207.5	*29.8*	127.7
	Total	73.5	232.2	80.6	158.2	73.9	231.2	78.9	156.5	80.1	234.8	91.9	163.1	64.0	117.8	83.7	126.3
Utah	Cities	*	*	*	*	*	*	*	*	244.3	91.2	*140.0*	227.3	186.0	79.4	*75.0*	181.0
	Rural	*	*	*	*	*	*	*	*	390.7	97.1	*33.3*	338.9	312.7	71.6	*66.7*	274.2
	Total	*	*	*	*	*	*	*	*	339.4	94.7	*81.8*	297.8	265.4	74.8	*72.4*	238.5
Vermont	Cities	109.3	158.4	*100.1*	147.9	109.4	153.0	*100.0*	147.5	114.7	147.8	*100.0*	147.3	92.7	72.1	—	104.7
	Rural	121.3	138.4	*133.3*	147.2	110.7	135.5	*75.0*	134.8	114.3	134.7	*125.0*	136.6	96.5	91.3	*100.0*	111.5
	Total	119.9	142.5	*120.0*	147.3	110.5	139.0	*77.7*	136.7	114.4	137.3	*116.7*	138.2	95.9	86.5	*66.7*	110.4
Virginia	Cities	*	*	*	*	*	*	*	*	163.1	163.6	91.6	170.7	115.6	101.5	71.6	117.4
	Rural	*	*	*	*	*	*	*	*	255.3	125.4	159.2	252.6	200.7	103.6	137.0	200.1
	Total	*	*	*	*	*	*	*	*	233.4	144.7	139.2	232.6	177.7	102.4	117.1	176.8
Washington	Cities	*	*	*	*	*	*	*	*	169.1	123.2	*63.0*	184.8	132.2	84.3	*57.6*	140.3
	Rural	*	*	*	*	*	*	*	*	201.5	116.2	*42.3*	203.8	168.4	91.7	*56.8*	168.0
	Total	*	*	*	*	*	*	*	*	286.6	119.9	58.5	194.8	150.1	87.5	57.4	153.6
Wisconsin	Cities	*	*	*	*	*	*	*	*	178.4	142.2	75.0	194.8	143.2	115.9	*68.2*	156.9
	Rural	*	*	*	*	*	*	*	*	266.0	57.6	37.1	209.1	217.9	57.5	*65.2*	186.9
	Total	*	*	*	*	*	*	*	*	231.5	89.8	60.4	203.6	187.9	81.6	67.2	174.8
Totals	Cities	100.5	267.5	93.1	181.7	100.5	247.8	89.2	172.5	117.0	228.3	79.6	173.0	93.2	166.9	66.8	132.0
	Rural	141.1	215.4	82.5	179.0	137.7	199.9	109.0	170.1	177.7	156.5	146.2	187.4	144.8	118.8	118.4	150.8
	Total	117.8	252.4	91.4	180.7	116.3	234.1	94.2	171.6	148.1	205.2	114.3	179.8	118.8	151.8	93.7	140.6

* Not in the Birth Registration Area in designated year.

In this state the native population, even when so broadly defined as to involve necessarily only one full generation in this country, did not, in any of the four years reviewed, come within a long distance of reproducing itself. It produced only about 0.8 of a birth for each death. Biologically that situation can have but one end.

The District of Columbia is in many respects demographically unique. Its abnormality is well reflected in the vital indices. *A*, while absolutely small, is still larger than *B* in each of the four years. The foreign population of the District succeeded in reproducing itself in only one of the four years, 1917, and then only barely so. Because of the obvious peculiarities of the population of the District no particular significance attaches to the result either way.

In Indiana, Kansas and Kentucky, in the two years for which data are available, the native population exhibits greater vitality than the foreign. (Index *A* greater than *B*.) The only exception is in Indiana cities, where the usual relation of *B* greater than *A* prevails. The foreign population makes a particularly bad showing in the rural portions of these states. It is by no means wholly a question of smaller numbers involved. For example in 1917, 4066 foreign whites died in Indiana, and only about 2000 more (exactly 6305) died in Connecticut. But whereas there were but 4952 births of which both parents were foreign in Indiana, there were 20,341 such births in Connecticut. The Indiana foreign population, in respect of racial stamina, age distribution and otherwise, is a less fit and vigorous population than the Connecticut foreign population. What is of more interest is the comparison of Vital Index *A* for these three middle western states with *A* for Connecticut. We shall return later to a more particular discussion of this, merely noting now that Index *A* is much higher in the middle west native population.

Maine is like Connecticut in the general trend of its figures for Index *A* and Index *B*. So also are the other New England states, Massachusetts, New Hampshire, and Rhode Island, with differences in degree in the several states. Vermont is the only New England state having Index *A* generally larger than 100.

In Maryland the native white population is reproducing itself less effectively in the cities than the foreign white, but much more effectively in the rural districts, making for the whole state a net balance in respect of biological vitality in favor of the native.

Michigan shows the same relation as Maryland as between urban and rural, but the net balance of the total, owing to the distribution

TABLE XVII.

States generally having

A less than 100.			*A* greater than 100.			*B* less than 100.			*B* greater than 100.		
Urban.	Rural.	Total.	Urban.	Rural.	Total.	Urban.	Rural.	Total.	Urban.	Rural.	Total.
Conn.	Conn.	Conn.	D. C.	Ind.	D. C.	D. C.	Ind.	D. C.	Conn.	Conn.	Conn.
Maine	Mass.	Maine	Ind.	Kan.	Ind.	Ky.	Kan.	Kan.	Ind.	Maine	Ind.
Mass.	N. H.	Mass.	Kan.	Ky.	Kan.	N. C.	Ky.	Ky.	Kan.	Mass.	Maine
N. H.	R. I.	N. H.	Ky.	Maine	Ky.	Utah	Md.	Utah	Maine	Mich.	Md.
N. Y.		N. Y.	Md.	Md.	Md.		Utah	Wis.	Md.	Minn.	Mass.
R. I.		R. I.	Mich.	Mich.	Mich.		Wis.		Mass.	N. H.	Mich.
			Minn.	Minn.	Minn.				Mich.	N. Y.	Minn.
			N. C.	N. Y.	N. C.				Minn.	N. C.	N. H.
			Ohio	N. C.	Ohio				N. H.	Ohio	N. Y.
			Pa.	Ohio	Pa.				N. Y.	Pa.	N. C.
			Utah	Pa.	Utah				Ohio	R. I.	Ohio
			Vt.	Utah	Vt.				Pa.	Vt.	Pa.
			Va.	Vt.	Va.				R. I.	Va.	R. I.
			Wash.	Va.	Wash.				Vt.	Wash.	Vt.
			Wis.	Wash.	Wis.				Va.		Va.
				Wis.					Wash.		Wash.
									Wis.		

of the population between urban and rural, is in favor of the foreign.

Minnesota, Utah, Washington, and Wisconsin exhibit in varying degrees the same phenomenon we have seen in Indiana, Kansas and Kentucky. The native population is reproducing itself more effectively than the foreign, both in urban and rural communities.

New York, Ohio and Pennsylvania behave in general like the New England states, the foreign populations in varying degree exceeding the native in net vitality.

North Carolina and Virginia, the only southern states included in Table XVI, have small foreign populations, especially the former. The native population is on the whole definitely more effective in net vitality than the foreign.

Summarizing, we may say that in the New England states (with the exception of Vermont) and New York the native population is not reproducing itself, and in Vermont only barely so. The native rural population of New York State is just about breaking even between births and deaths. The foreign population is practically everywhere showing a higher degree of vitality except in Kansas, Kentucky, Utah and Wisconsin. Also in the rural portions of Indiana and Maryland their vital index falls below 100. The general facts leaving out the influenza year 1918 are summarized in Table XVII.

The relation between urban and rural population in respect of the vital index is interesting. So far as the white population is concerned, we note, having regard for indices *A* and *B*, that the following rules hold generally. The native population has a *lower* vital index in cities than in rural districts: the foreign population shows the reverse relation, the *higher* index being for the city populations. By and large, and with all factors included, as they are in the vital index, it appears that the foreign population, as it is actually constituted in the B. R. A. in respect of age, etc., is a biologically fitter population in the cities than is the native; while the native population is better under rural conditions.

A point might well be made here by a critic of the preceding results. He might say: " It is all very well to show that the foreign population, as actually constituted in this country, shows a higher vital index than the native, but this fact may only mean that the foreign population is more favorably distributed in respect of age than the native, both in regard to deaths and to reproduction. If the foreign population contains a great many more people than the native population in early adult life, which is the period of a higher reproduction (birth) rate and a low death rate, all the results you have shown might well appear from this cause alone.''

This is an entirely valid criticism so far as any evidence we have yet presented goes. The vital indices of Table XVI are crude indices. We need age-specific vital indices for native- and foreign-born populations.

Let us put the matter in this way: Suppose that a gigantic corral were constructed with two compartments. Suppose that further there were put into one of these compartments, on a given date, all the native-born women aged 20–24 inclusive say, while into the other compartment were put all the foreign-born women in the country of the same ages. Suppose them all to be told that they were to stay there for one year, but that men could have free access to the corrals for purposes of reproduction. Finally suppose that similar corrals were constructed, and the women impounded in them, for each age group from say 10–14 at one extreme to 55 and over at the other extreme.

In any one compartment of any one corral during the year (*a*) some of the women would have babies, and (*b*) some of the women would die. If we kept statistical record of these events we could, at the end of the year, calculate the age specific vital index for each group of women. It would not be the general population vital index because no male deaths were included (and cannot be because of lack of published data). But it would be an age-specific vital index for the females as reproductive units.

TABLE XVIII.

Age specific vital indices for native-born and foreign-born women in B. R. A. 1919.

Ages.	Births from mothers born in U. S.	Deaths of native-born females.	Vital indices for native women.	Births from foreign-born mothers.	Deaths of foreign-born females.	Vital indices for foreign women.
10–14 ...	391	5,002	7.82	15	268	5.60
15–19 ...	77,048	7,763	992.50	10,768	759	1418.71
20–24 ...	258,876	11,854	2183.87	74,247	2,120	3502.22
25–29 ...	250,548	13,189	1899.67	102,429	3,317	3088.00
30–34 ...	166,777	11,813	1411.81	83,326	3,583	2325.59
35–39 ...	101,638	10,603	958.58	56,414	3,723	1515.28
40–44 ...	33,832	9,511	355.71	18,878	3,566	529.39
45–49 ...	3,202	10,092	31.73	1,866	4,120	45.29
50–54 ...	68	10,926	.62	54	4,968	1.09
55 and over..	26	96,919	.03	13	47,478	.02
Totals	892,406	187,672	....	348,010	73,902	....

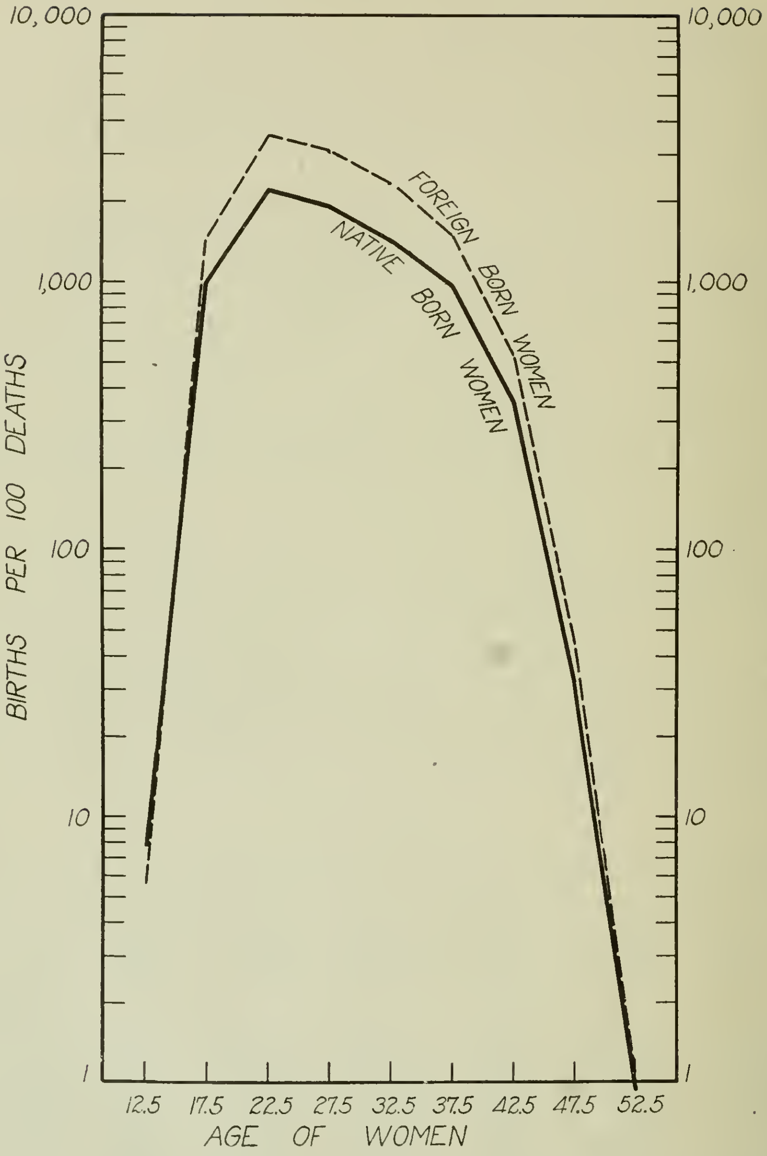

FIG. 14. Showing the differences in specific vital indices for native-born and foreign-born women in 1919. Solid line, native-born women; dash line, foreign-born women.

The results of exactly such an experiment for the women of the B. R. A. in the year 1919 are shown in Table XVIII.

The figures in Table XVIII show plainly enough that at every age between 15 and 54 inclusive the foreign-born women have higher *specific* vital indices than native-born women. How much so is shown graphically in Fig. 14.

As a reproductive machine the foreign-born woman far excels the native-born. For each native-born woman dying between 20 and 24 years of age, the native-born women as a group produce approximately 22 babies. But for each foreign-born woman dying between 20 and 24, the foreign-born women as a whole produce 35 babies. It is in these five years that women, under conditions of life as now socially organized in the United States, do their best work biologically for the race, " best " being taken here in the sense of biological efficiency and economy.

So far as I am aware no attempt has been made hitherto to calculate age-specific vital indices. They picture, as exhibited in Table XVIII and Fig. 14, an extremely interesting biological fact. If we had such indices for populations of lower animals in different environmental situations, we should be in a position to know a great deal more than we now do as to the method of evolution. For it is the net balance between births and deaths which is the most significant information that can be had about the progress of the struggle for existence.

It may be objected in Table XVIII that we have put all births (both male and female) against only female deaths. The thought in doing this was that, after all, females have to produce *all* the babies whether the latter are boys or girls. If one wishes to postulate the problem in this way: how many new reproductive machines (females) do women of a specified age produce as a class for each similar reproductive machine lost by death, then of course one should take only female births in computing the specific vital indices. The result would be, of course, that the births and consequently the indices, Table XVIII, would be about one half as large absolutely as they are in that table, but the general *form* of the curve of Fig. 14 would be unchanged. What would be of great interest, but which we now unfortunately cannot do, is to make a table corresponding to Table XVIII, but for *males* instead of females. Why we cannot do it is because the birth statistics published do not separate fathers by both age and nativity. We can, however, get fathers of all nativities by age, and from that see how the curve of age-specific vital indices runs for males in general, as compared with that for females.

The data for male age-specific vital indices are given for 1919 in Table XIX.

TABLE XIX.

Age specific vital indices for men in B. R. A. 1919. (Births per 100 deaths.)

Ages.	Births.	Deaths.	Vital index (births per 100 deaths).
10–14	15	7,086	.21
15–19	14,211	11,395	124.71
20–24	190,061	14,804	1,283.85
25–29	338,605	17,685	1,914.65
30–34	313,294	19,136	1,637.20
35–39	239,578	20,401	1,174.34
40–44	131,025	18,666	701.94
45–49	65,728	20,807	315.89
50–54	20,627	22,032	93.62
55 and over	10,123	163,447	6.19
Totals	1,323,267	315,459	......

The striking differences between the vital indices of Table XIX and those of Table XVIII are the higher values for males than females in the ages after 25 or 30, and the lower values earlier. The male specific vital index curve, as shown in Fig. 15, is flatter topped and more spread out in general than the female curves. This is what would be expected from the known facts about the male mortality and reproductive capacities relative to age.

With the data for specific vital indices in hand the obvious thing to do would be to proceed to the calculation, for each state and the B. R. A. as a whole, of vital indices *corrected* for the age distributions of the several populations. Unfortunately, however, in this case as in so many others, we are balked in the performance of this desirable enterprise because the raw materials are not so tabulated in official publications as to make it possible. We shall have to resort to indirect methods to check up with more critical care the conclusions we have drawn from the crude vital indices in Table XVI.

The chief conclusions to which we have come so far from an examination of vital indices are these:

First, that generally index *B* (foreign population) is higher than index *A* (native population).

Second, that generally index *A* is higher for rural than for urban populations.

Third, that generally index *B* is higher for urban than for rural populations.

We have now to ask this question: are these differences such in direction and magnitude as might reasonably be expected to arise merely from differences in the age constitutions of the several populations involved?

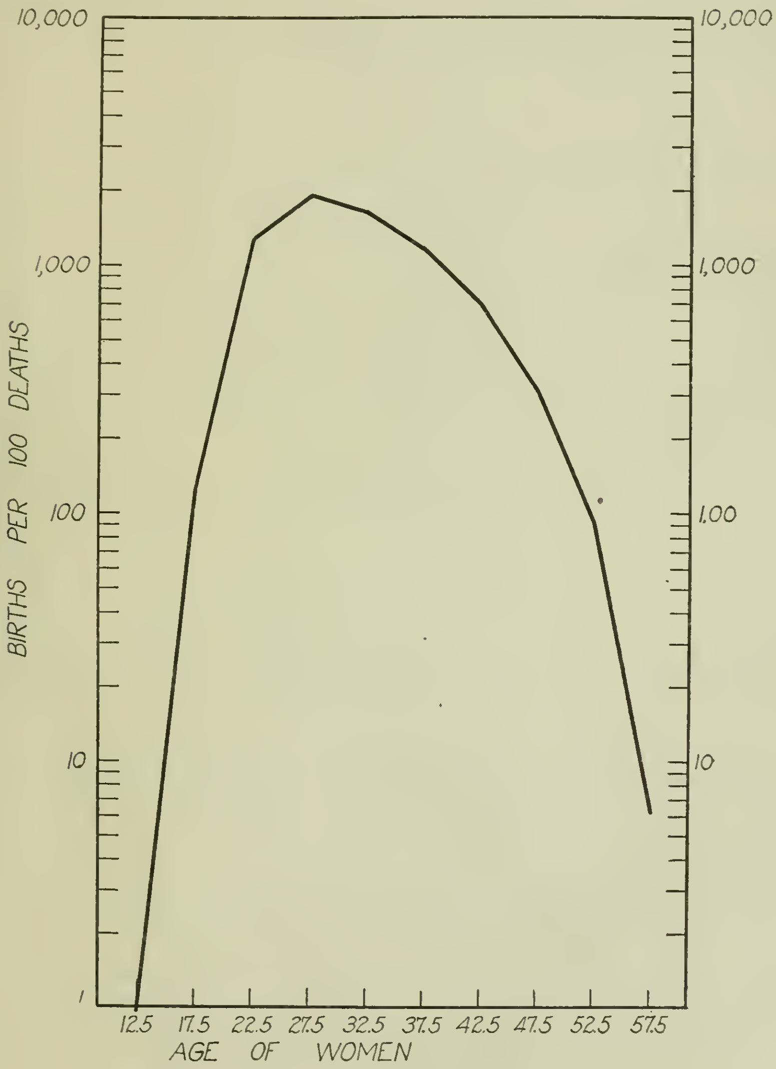

FIG. 15. Age-specific vital indices for males (see text).

As the first step in answering this question Table XX is presented. This shows for each state in the B. R. A., in 1917 and 1918, the percentage of women of ages 15 to 44 inclusive in the following divisions of the population in 1910: (*a*) urban native-born white, (*b*) rural native-born white, (*c*) urban foreign-born white, (*d*) rural foreign-born white. The ages 15 to 44 were chosen because Table XVIII shows that it is only within these years that the female age-specific vital indices rise to values greater than 100.

TABLE XX.

Percentage of women 15–44 years of age in the population in 1910.

State.	Order.	Native urban.	Order.	Native rural.	Order.	Foreign urban.	Order.	Foreign rural.
Connecticut	19	45.0	20	38.8	2	64.7	4	58.1
District of Columbia	4	52.7	......	......	15	54.5	......	......
Indiana	6	52.4	5	45.3	17	49.2	20	31.4
Kansas	7	52.3	1	46.3	20	46.6	16	40.6
Kentucky	3	53.8	6	44.4	21	34.2	19	32.0
Maine	20	44.9	16	40.9	8	61.3	6	56.0
Maryland	12	50.8	4	45.5	16	54.0	12	46.5
Massachusetts	18	45.3	19	40.1	3	64.4	3	58.8
Michigan	11	51.1	11	43.7	13	55.4	13	44.1
Minnesota	2	54.3	8	44.3	11	58.1	14	42.4
New Hampshire	21	42.9	18	40.3	4	63.4	5	57.2
New York	15	48.0	10	43.7	1	64.8	10	48.9
North Carolina	10	51.4	12	43.3	9	58.7	9	52.1
Ohio	8	52.2	7	44.4	14	55.0	15	41.9
Pennsylvania	14	48.6	14	42.1	7	62.3	2	63.8
Rhode Island	17	45.4	17	40.5	6	62.5	1	66.1
Utah	13	50.4	13	43.1	19	46.9	17	39.5
Vermont	16	46.3	15	41.6	12	56.0	11	47.1
Virginia	5	52.5	9	43.8	10	58.1	8	54.3
Washington	1	54.9	2	45.7	5	62.8	7	54.5
Wisconsin	9	52.0	3	45.6	18	48.6	18	36.6

From this table it is seen that, with some few exceptions, the following relations generally hold: The most favorable population in respect of the age distribution relative to vital index of females is the foreign urban, next comes the native urban, next the foreign rural, and last the native rural.

Turning now to our first conclusion we have the following question: if the proportion of females 15–44 were the same in the native-born population that it is in the foreign-born population would the former population, in any of its divisions, show as high values for the vital index as the latter population actually exhibits? The answer at once is that it could not, because Table XVIII shows that in each age class between 15 and 45 foreign-born women have a higher specific vital index than do native-born women, and if these indices were multiplied by the same population numbers the latter group would be bound to show a lower average vital index as a group. In other words, the foreign-born population has *both* a more favorable age distribution for the vital index, and also higher specific indices than the native-born.

The next question is: is the higher value of Index *A* for the rural native than the urban native population due to a more favorable age distribution of the former? As an aid to answering this question Fig. 16 is presented.

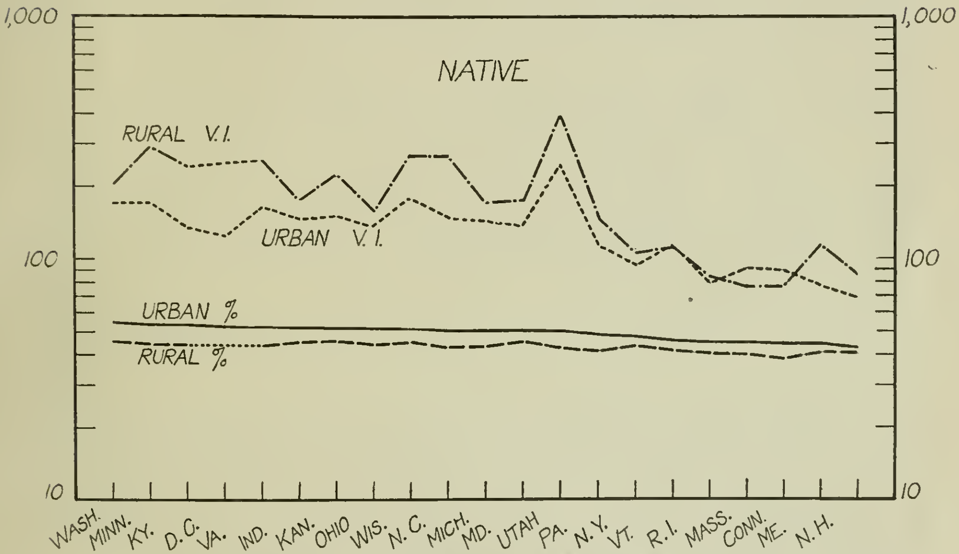

FIG. 16. Showing (*a*) percentage of native-born women 15–44 in urban population, (*b*) percentage of native-born women 15–44 in rural population, (*c*) Vital Index *A* for urban population, (*d*) Vital Index *A* for rural population.

The figure shows, first, the percentage of native women in the age period 15–44 inclusive in the urban population, the states being arranged in descending order of magnitude of this percentage. Next is plotted the percentage of this class of women in the rural population. It will be seen that in general this line runs parallel to, and everywhere *below*, the first. In other words, the age distribution of the rural population is, in every state included, distinctly less favorable to a high value of the vital index than is that of the urban native population.

Against these percentages taken from Table XX are plotted urban and rural values of Vital Index *A* for the same states, taken from Table XVI. The striking thing at once apparent is that, with the exception of the three states Vermont, Massachusetts, and Connecticut, the urban line lies everywhere *below* the rural, by an amount generally substantially in excess of that which separates the percentage lines. In other words, we see that while the native urban percentage of females in the child-bearing ages is higher than the native rural percentage, and would therefore lead to the expectation of higher vital indices in the former population, actually the vital indices show the opposite relation. In view of these facts there can be no doubt of the justice of our conclusion that rural conditions are substantially more favorable to the biological vitality of the native population than are urban conditions. Or, put in another way, the native population appears to be better adapted biologically to country than to city life.

The data are plotted on a logarithmic scale in order that slopes of the lines may be comparable. It is seen that in general the four lines run, in respect of their slopes, about parallel from the left margin of the diagram to and including Maryland. Utah's vital indices are much higher than would be expected from the age distribution of the child-bearing population alone. After that point clear to the right edge of the diagram, the relation between age distribution of the population and the values of the vital indices is obviously of a different order from that exhibited in the first half of the diagram. The New England states, for these are the ones here included, together with Pennsylvania and New York, have much lower vital indices for their native populations than the proportion of child-bearing women would entitle them to, on the basis of the showing of the other states. This can only mean that in the New England states especially, and to a less degree in New York and Pennsylvania, the age-specific vital indices of the native-born women must be extremely low. The native populations of these states are in a biologically unhealthy condition.

The next question is: is the general superiority of the foreign population in respect of the vital index in urban, as compared with rural environments, chiefly due to a more favorable age distribution of the child-bearing population in the former case?

Figure 17 puts the available data in graphic form.

The data plotted in this figure are the same, *mutatis mutandis,* as those in Fig. 16. The results, however, are quite different. Here the urban vital index line lies generally above the rural. In other words, it is apparent that a substantial part of the superiority of the foreign vital index under urban conditions must be in fact merely due to the more favorable age constitution of the urban as compared with the rural foreign population. Probably in most of the states nearly all of the superiority is due to this factor. It is, however, of interest to note that the vital index lines in Fig. 17 do not parallel throughout their course the age percentage lines, in respect of their slopes. The last 8 or so states at the right end of the diagram show generally lower vital indices, both urban and rural, than would be expected from the age constitution of the population, on the basis of the other states included in the diagram. These are middle western states for the most part, where the foreign-born population, while socially more desirable perhaps than the foreign-born population of the more highly industrialized eastern states, is nevertheless a biologically less vigorous population.

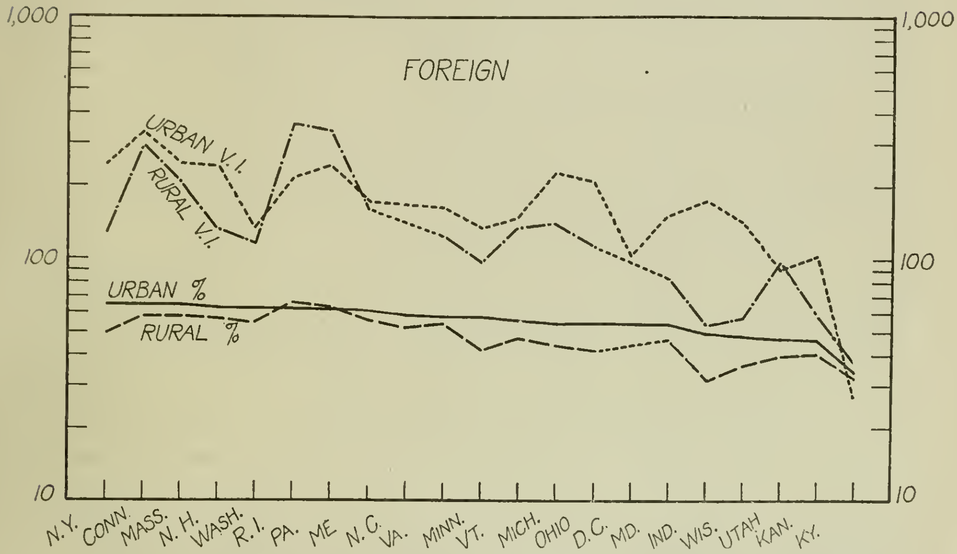

FIG. 17. Showing (*a*) percentage of foreign-born women 15–44 in urban population, (*b*) percentage of foreign-born women 15–44 in rural population, (*c*) Vital Index *B* for urban population, (*d*) Vital Index *B* for rural population.

The question of the secular trend of the vital index of the several components of our population is of great interest, but cannot be satisfactorily dealt with for two reasons: first, because only four years are available and one of these is highly abnormal because of the influenza epidemic; and second because the B. R. A. changed its composition radically by addition in the middle of the period. In consequence of this latter point the totals for 1917 and 1918 are not comparable with those for 1915 and 1916. When data have accumulated for a larger number of comparable years it will be profitable to go into the problem in detail. The writer hopes then to return to the matter.

There is one point of interest in the general connection discussed here, namely, the effect of the influenza epidemic in the vital indices. Comparing the totals of 1918 with those of 1917 it appears that:

Vital Index *A* (native white population) for cities fell 23.8 points or 20 per cent. of its value in 1917.

Vital Index *A* (native white population) for rural areas fell 32.9 points or 19 per cent. of its value in 1917.

Vital Index *A* (native white population) for total areas fell 29.3 points or 20 per cent. of its value in 1917.

Vital Index *B* (foreign white population) for cities fell 61.4 points or 27 per cent. of its value in 1917.

Vital Index *B* (foreign white population) for rural areas fell 37.7 points or 24 per cent. of its value in 1917.

Vital Index *B* (foreign white population) for total areas fell 53.4 points or 26 per cent. of its value in 1917.

From these figures it is evident that the epidemic reduced the net vitality of the population from 1/5 to 1/4 of what it was in the preceding year. This was a sharp blow. Further it appears that rural populations were slightly, but probably not significantly, less damaged relatively, in a biological sense, than urban populations. The foreign population was definitely harder hit biologically than the native. This might reasonably have been expected from all that we know of the general hygienic ignorance of this element of the population.

Let us now turn to the examination of Vital Index *C* for the negro population in comparison especially with *D* (total white population) and *A* (native white). The material includes only three southern (or border) states with large negro populations, namely North Carolina, Virginia and Maryland. In a number of the northern states, however, the negro population is large enough to furnish births and deaths in sufficient magnitude to give reliable indices.

The immediate and striking thing which our indices exhibit in comparing *C* and *D* is the low value of the former compared with the latter. Indeed one may say generally that, except in the rural districts of the southern states, practically never does the vital index of the negro population rise to a value of as much as 100. But plainly enough any population with a vital index under 100 is a dying population. Such a population is bound, in the fullness of time, to disappear completely, if nothing whatever is done about the case. Nowhere in *cities,* even in the southern cities, does the value of the negro vital index get to 100 in a fairly normal year such as 1917.

Even in rural portions of the B. R. A. the negro index does not approach in magnitude the total white index nor the native white index for the same communities. This is true in southern states as well as in northern. It would be difficult to find a more complete and critical demonstration than that furnished by these indices of the fact that the negro is biologically a less fit animal, *in the American environment physical, social and general,* than the white. It is idle to argue that if the negro were given a better chance, in a broad environmental sense, he might exhibit a degree of biological fitness, as measured by the vital index, equal or superior to that of the whites. Nobody knows whether that is true or not. And the important things are the facts as they are. Under conditions *as they are,* Nature, by the slow but dreadfully sure processes of biological evolution, is apparently solving the negro problem in the United States, in a manner which, when finished, will be like all of Nature's solutions, final, complete and absolutely definite. Just in direct proportion as the negro becomes anything but an agricultural laborer in the southern states does he hasten the time of his final extinction in this country. If he elects, which he will not, to become *exclusively* a farm laborer in the southern states, either for himself or somebody else, he must strictly limit his birth rate, which again he will not do, to the point where the numbers of his population will not exceed the economic demand and necessity for that particular kind of population, namely negro farmers. It would appear that, by nature, the negro is biologically fitted only to be in this country just precisely what he was before the Civil War. namely, an agricultural laborer in the South. This does not mean, of course, that there is any biological justification for the institution of slavery. On the contrary, as the South learned to its bitter cost, slavery is a most unstable and unbiological institution. But subtract slavery from the issue, as it happily has been subtracted, and the certainty of the biological conclusion cannot be shaken, so far as the experience of the last 50 years may be taken as indicative, and that is a fairly long time.

It is of interest to examine the comparative effect of the influenza epidemic on negro and white populations. Taking totals as before for 1918 as compared with 1917 we have:

Vital Index *C* (negro population) for cities fell 12.8 points or 16 per cent. of its value in 1917.

Vital Index *C* (negro population) for rural areas fell 27.8 points or 19 per cent. of its value in 1917.

Vital Index *C* (negro population) for total areas fell 20.6 points or 18 per cent. of its value in 1917.

Vital Index *D* (total white population) for cities fell 41.0 points or 24 per cent. of its value in 1917.

Vital Index *D* (total white population) for rural areas fell 36.6 points or 20 per cent. of its value in 1917.

Vital Index *D* (total white population) for total areas fell 39.2 points or 22 per cent. of its value in 1917.

The figures show that biologically the negro population of the B. R. A. was distinctly less severely damaged by the epidemic than the white population. The relation between urban and rural areas is exactly the reverse for negroes of what it is for whites. The rural negro population index was reduced more by the epidemic than the urban negro index, both absolutely and relatively.

VII.

Conclusion.

Out of the mass of statistical detail which has been presented in the preceding sections what sort of a general picture emerges? Have we answered, on the basis of vital statistics, our initial question as to what *kind* of a population this country is likely to harbor in the future, near or remote?

It must be regretfully stated that we have not been able to answer this question in any final or particularly satisfactory manner. I think, however, that it may fairly be said that we have shown that the responsibility for this failure is not primarily ours. It arises, as has been specifically pointed out in many places, because in the first instance the existing official information is not tabulated in such a way as to make possible many of the inquiries we should like to undertake and which are absolutely essential, if we are ever to have any deep understanding of our demography. In the second place, some information which is needed is not collected at all.

So far as the first of these difficulties is concerned, I am of the opinion that but one solution is practically feasible or possible. It is for some private foundation or group of individuals, who may be sufficiently interested, to come forward and finance an investigative organization for a period of years, to work in cooperation with the Census Bureau (a cooperation which the Census Bureau would probably gladly enter into). What this organization would do would be, in the first place, to tabulate in various new ways the raw data as to births and deaths, and in the second place proceed, after the preliminary tabulations were finished, to an exhaustive biometrical analysis of the data. The project is, from the standpoint of expense, by no means an impossible one, and I can think of no investigation likely to have such far-reaching anthropologic, eugenic, social, and even perhaps economic consequences, as this would. It is as true today as it was a quarter of a century ago when the eminent statistician, Dr. G. B. Longstaff (15), said in a meeting of the Royal Statistical Society, devoted to a discussion of the official statistics of the United States, that:

> As a theater of statistical investigation at the present time, no country in the world was so interesting as the United States, but with the imperfect tools at command they were unable to deal with a number of problems, quite unequalled elsewhere for magnitude and interest

This is not the place to present detailed plans for such an organization as that suggested, whether for its budget or its specific problems. The most I can hope for is that the discussion set forth in these pages may excite sufficient interest in the whole matter to make it profitable to outline specific plans for a real investigation.

Incomplete as our picture is as compared with what we should like to have, it still is a fact, I think, that the material which has been presented in these pages does give a little clearer and more comprehensive view than we have hitherto had of present tendencies, working in that great struggling mass of more than one hundred millions of individuals, who collectively make up the soul and body of these United States. Let us endeavor to summarize briefly what our findings are.

In the first place our population has indubitably passed the point of inflection of its growth curve, and from now on we may confidently expect an ever-increasing pressure of population on the means of subsistence, to use Malthus's classical phrase. The vital question is what kind of people are to inherit the blessings which Providence has so lavishly bestowed upon our portion of the North American continent? All cannot have them. There is not room enough. Man still breeds so fast that at all too frequent intervals he resorts to force to determine whether his or some other particular kind of people are going to enjoy the pleasures of continued existence.

The struggle may be varied in its manner. Economic pressure may in the long run be as effective an eliminant as machine guns. In general, unless forcibly prevented—which means finally by murder and sudden death—that people will inherit the earth and the fullness thereof which has habitually the highest vital index. The advocate of birth control as a solution of the problem of population should remember this, and draw from it the logical conclusion that if, for any reason whatever, he does not want the people who have the highest vital index to be the inheritors, he must be prepared to do something a good deal more violent than merely to control the birth rate of *his* own kind of people which is, in practical effect, about all that he has done so far. And he must not forget that people who have a high vital index are apt as a group to be pretty good fighters, in a technical military sense.

In the United States at the present moment certain broad facts seem to be outstanding in their significance for the future. They are:

1. That newly arrived foreigners rather speedily fuse effectively with the stocks already here, to a degree much greater than is presupposed, at least in most popular discussions of the subject of immigration and related matters. The temperature of the melting pot is higher than we generally give it credit for being. It really melts to a definite and significant degree.

2. That immigrant stocks are in the stark, raw business of reproduction vastly superior to native stocks, even to native stocks which have been here only a generation or two. Beyond this point there is no definite evidence, but all that can be inferred from the meager data available seems to indicate that it is a general rule that the farther we get in the fusion process from pure fresh immigrant stock the lower the vital index becomes. The end of the series is represented by the native stock of New England which is simply passing away, with considerable rapidity.

3. That native stock does better, in a biological sense, under rural than under urban conditions, while the opposite is true for immigrant stock.

From the standpoint of *quality* of the population in respect of health, intelligence, and all round efficiency, it is suggested that the wisest policy from now on for the country to pursue would be one which might be called intermittent or periodic immigration. Let all immigration of any sort whatever be rigidly and completely prohibited for a period of say 20 years, to be followed by a period of say 10 years of free immigration, with only such restrictions physical, economic and criminal, as we have in the immediate past imposed. During the period of no immigration the foreigners of the last influx and their children would become sensibly assimilated and fused into the American population. For the most part, they would have become Americans in a real sense of the word. Such a plan would further provide for that periodic inflow of new blood, of low living standards, which seems to be both an economic and social necessity, at least to civilizations which are still in the stage of industrial development and exploitation. Finally such a plan would, so far as one can foresee, postpone the period of pressing over-population as long as it can humanly be postponed, without the necessity of resorting to internal interferences of dubious practicability.

The United States has been, from its beginning, a gigantic experiment in human genetics. The experiment has gone on now three centuries, roughly speaking. When I compare the net result, as indicated by the human product now here—the product counted in the 1920 census—with the much more nearly pure-bred peoples of Europe, I cannot force myself to take that pessimistic outlook that I am told by some of my " 100 per cent. Nordic " friends that I ought to. It seems to me, looking at the matter as a biologist, that a real, distinct, unique *American* people has evolved in the course of the experiment and is still continuing to evolve. Further, as people go, it is not a bad people. Its most interesting and valuable feature is that it is still changing and evolving. I can find no manner of bitter feeling or even regret that the pure English-Scotch-Welsh stock of the original settlers will eventually not be the dominant element in the complex of Am ican germ-plasms that it has been in the past, though persona wholly of that stock, and am, for three centuries, an Americ a state of affairs was, in the natural course of events, bound to come about. There cannot, by any possibility whatever, be anything approaching biologically pure race stocks in this country a century hence. There are practically none now, with the exception of the Jews, and there is every present reason to believe that even they will be far less pure in 2021 than they are in 1921. How far we have progressed in only about one generation, in point of time, in racial fusion, is indicated by the comparison of a careful estimate of the racial stocks in the country in 1872 with conditions now existing. In the Journal of the Royal Statistical Society in 1872 (16), it was estimated that the following figures gave the percentages of the different race stocks in the population at that time:

	Per cent. in population
Original British Stock	46
Irish	16
German	13
African (negro)	12
Recent British	8
French, Spanish and others	5
	100

The kind of people who will survive and run the affairs of the country, say a couple of centuries hence, when population pressure will be intense, will, I think, not be Englishmen, or Slavs, or Jews, or Italians, but *Americans*, of that type which has shown the greatest adaptability to the problems which life in this part of North America has presented. I think they will be just as gentle, as high-minded, as clever, as honorable, and as independent as any people on the face of the earth.

VIII.

SUMMARY.

1. It is shown that the population of the United States (*a*) is growing according to a curve of the form

$$y = \frac{be^{ax}}{1 + ce^{ax}},$$

(*b*) has passed the point of inflection of this curve, and (*c*) is rapidly approaching its asymptote.

2. The problem of the future is the *quality* of the asymptotic population. To estimate this from past conditions is one of the purposes of this paper.

3. It is shown that there are three general criteria by which the biological attributes of a population may be estimated or measured. These are: (1) somatic physical, (2) somatic psychological, and (3) biostatistical. These are defined and discussed. This paper deals

solely with the third of these criteria, including marriages, divorces, births, deaths, and the birth/death ratio.

4. Racial assortative mating in marriages effective in producing offspring in 1919 is discussed.

5. For the entire B. R. A. the percentage of amalgamation or fusion of foreign-born stocks with native-born, as compared with racially like effective matings of all sorts, as indicated by effective marriages in 1919, is just under 11.5 per cent.

6. The amount of racial amalgamation or fusion going on in the several states of the Registration Area is proportional in the most direct and close way to the amount of foreign-born white stock in the local population.

7. The same result holds if the percentage of fusion matings of foreign with native stock is taken upon the base of pure native matings, instead of all pure matings as before.

8. In 10 out of 23 states there are *more* effective mixed matings of the amalgamating type (i.e., foreign × native) than there are of racially true foreign × foreign matings.

9. In the three states Massachusetts, New York, and Connecticut, there were in 1919 as many or more matings of the type foreign-born × foreign-born as there were of the type native-born × native-born, *effective in producing offspring.* In these three states as large or larger additions to the future population were made in 1919 by the foreign-born as by the native-born. Further, in three other states, New Hampshire, Pennsylvania and Maine, the effective foreign × foreign matings were more than half as numerous as the effective native × native.

10. The foreigner in this country is much more apt to marry an American-born person, if he does not marry one of his own race, than he is to marry some other foreigner not of his own race.

11. In 1919, there were in the B. R. A. 78 racially different types of mating effective in producing offspring, not separating sexes of either mated partners or offspring. 65 per cent. of the births were from native × native matings, 10 per cent. of the children had one parent native-born and the other foreign-born, leaving approximately 25 per cent. of the births having both parents foreign-born.

12. In those parts of the country where a relatively large proportion of the population is foreign-born, the fertility of the foreign-born women is greatly in excess of that of the native-born. Almost, if not

quite, the first biological result of Americanization is to reduce the fertility of marriages.

13. The illegitimate rate is higher per 1000 foreign-born women capable of having an illegitimate baby than it is per 1000 native born women in the same social situation. But per 1000 total births the illegitimate rate is smaller for foreign- than for native-born mothers. The explanation of this apparent paradox is given.

14. The stillbirth rate is higher, on the basis of total live births, for foreign- than for American-born women in general. In the case of Scandinavian, German, Polish and Hungarian mothers, the stillbirth rate is lower than for native-born mothers.

15. After infancy and early childhood the age- and sex-specific death rates are lower at all ages for native-born of native parents than for either native-born of foreign parents, or foreign-born. A detailed discussion of mortality and race stock is presented.

16. The biological vigor as indicated by the vital index (100 births/deaths) of the native-born population is much lower than that of the foreign-born population. In the New England states (except Vermont) and New York, the native population is not reproducing itself, and in Vermont only barely so.

17. The native population has a lower vital index in cities than in rural districts; the foreign population shows the reverse relation, the higher index being for the city population.

18. A study of age-specific vital indices for women shows that as a reproductive machine the foreign-born woman far excels the native-born. For each native-born woman dying between 20 and 24 years of age, the native-born women as a group produce approximately 22 babies. The corresponding figure for foreign-born women is 35.

19. The peak value of the vital index for women falls in the age group 20 to 24, inclusive. For men the peak value is in the period 25 to 29.

20. Age and sex corrected vital indices are discussed.

21. Rural populations were slightly, but probably not significantly, less damaged, relatively in a biological sense, than urban populations by the influenza pandemic. The foreign population was definitely harder hit biologically by the pandemic than the native.

22. Except in the rural districts of the southern states practically never does the vital index of a negro population rise to a value of as much as 100. Nowhere in cities, even in southern cities, does the value of the negro vital index get to 100.

23. Biologically the negro population of the B. R. A. was distinctly less severely damaged by the influenza epidemic than the white population.

24. Some eugenic bearings of the study are discussed.

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